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Dynamics of inner ear pressure change with emphasis on the cochlear aqueduct

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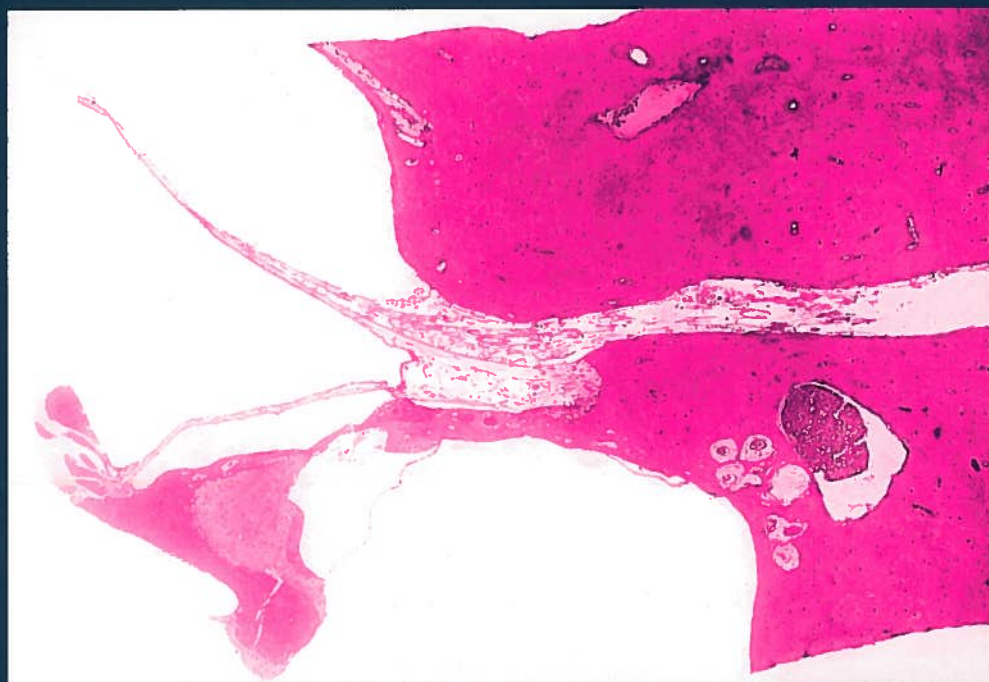
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Dynamics of Inner Ear Pressure Change with Emphasis on the Cochlear Aqueduct



Elisabeth O. Laurens-Thalen

**DYNAMICS OF INNER EAR PRESSURE CHANGE
WITH EMPHASIS
ON THE COCHLEAR AQUEDUCT**

E.O. LAURENS – THALEN

Stellingen
behorende bij het proefschrift

**Dynamics of Inner Ear Pressure Change
with Emphasis
on the Cochlear Aqueduct**

E.O. Laurens - Thalen

Groningen, 14 april 2004

1.

De stromingsweerstand van de aquaductus cochlearis is variabel.
(dit proefschrift)

2.

De ductus perioticus is verbonden met het ronde venster. Mede hierdoor beïnvloedt de stand van het ronde venster de stromingsweerstand van de aquaductus cochlearis.
(dit proefschrift)

3.

Bij een verhoging van de binnenoordruk neemt de stromingsweerstand van de aquaductus cochlearis af, waardoor een sneller drukherstel in het binnenoor kan optreden.
(dit proefschrift)

4.

Ductus perioticus is een onjuiste benaming voor de structuur in de aquaductus cochlearis. Een betere benaming zou zijn *rete perioticus*.

5.

Bij patienten met de ziekte van Menière ontbreekt vaak een nystagmus gedurende een aanval. Ook het ontbreken van een nystagmus gedurende een drop-attack bij deze patienten, pleit ervoor dat duizeligheid bij de ziekte van Menière met name wordt veroorzaakt door pathofysiologie ter plaatse van de utriculus en de sacculus.

6.

Ondanks het toenemende aantal vrouwelijke artsen is een ziekenhuis nog steeds een mannenmaatschappij. Dit wordt bevestigd door het feit dat er in geen enkel Academisch Ziekenhuis in Nederland een protocol bestaat voor zwangere artsen met richtlijnen voor de omgang met inhalatie-anaesthetica en chemotherapeutica.

7.

Het door een patient achterwege laten van de vermelding aan de behandelend arts dat hij of zij HIV positief is, is onverantwoord ten opzichte van het medisch personeel en andere patienten en hoort thuis in het wetboek van strafrecht.

8.

Zolang de overheid niet in staat is om het roken in instellingen voor gezondheidszorg rigoreus te verbieden, is de landelijke anti-rook campagne gedoemd te mislukken.

9.

Voor sommige patienten heeft een opname in het ziekenhuis veel overeenkomst met een verblijf op de camping. Ook in het ziekenhuis kan gebrek aan privacy en gemeenschappelijk gebruik van sanitaire voorzieningen leiden tot decorumverlies.

10.

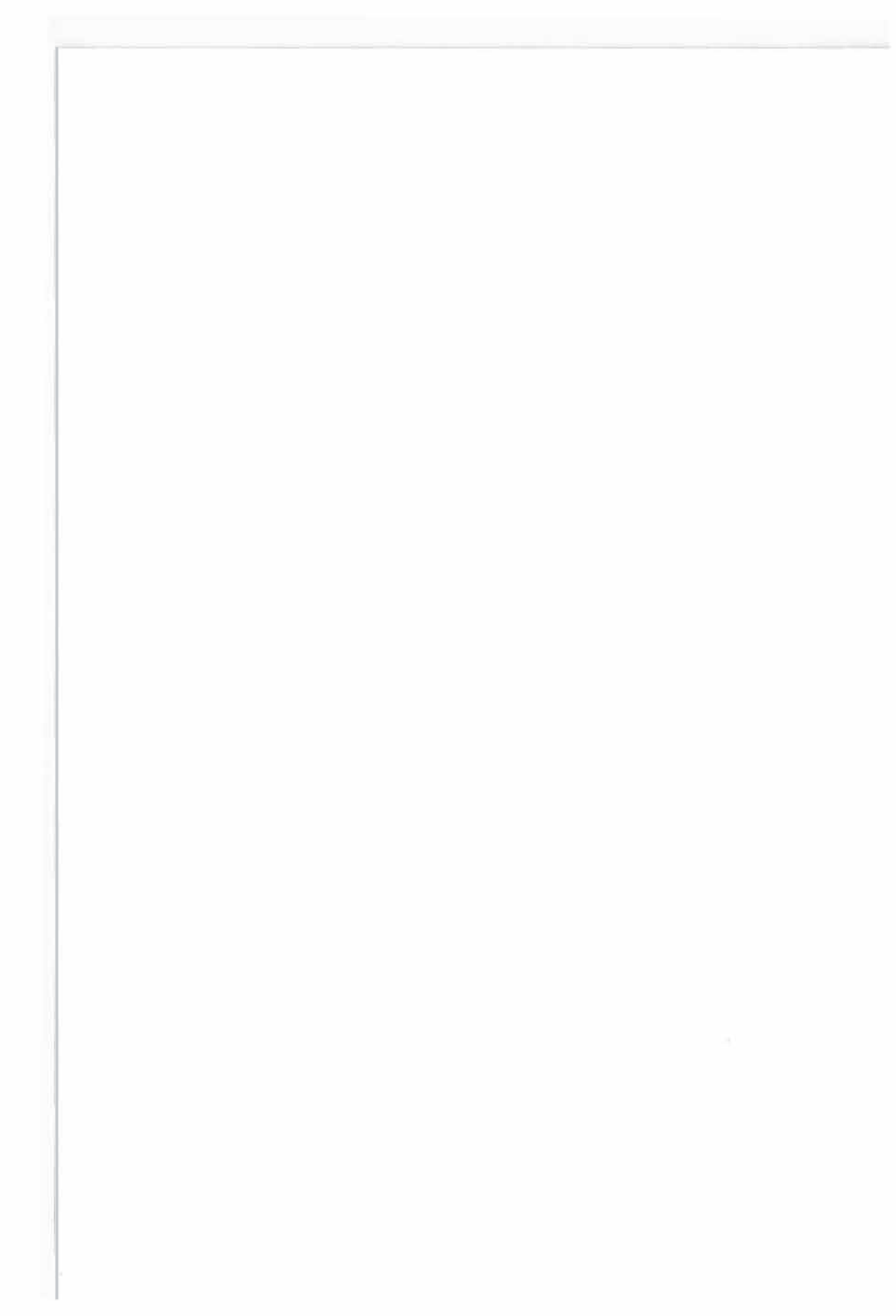
De dankbetuiging aan het thuisfront, zoals deze in vele proefschriften wordt verwoord, doet vermoeden dat het schrijven van een proefschrift vaak als excuus is gebruikt om zich te onttrekken aan de huiselijke verplichtingen.

11.

“Hou dichter bie kerk, hou loater der in.” (*Gronings gezegde*)

12.

Opheffing van het verbod om op de snelweg rechts in te halen kan een bijdrage leveren aan de vermindering van het file-probleem.



RIJKSUNIVERSITEIT GRONINGEN



**DYNAMICS OF INNER EAR PRESSURE CHANGE
WITH EMPHASIS
ON THE COCHLEAR AQUEDUCT**

PROEFSCHRIFT

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
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om 16:15 uur

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geboren 5 juni 1970
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Aan mijn ouders

Voor Roland, Govert
en Frederique

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Chapter 1

GENERAL INTRODUCTION

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General introduction

The inner ear or labyrinth contains the important sense organs for hearing and the maintenance of spatial equilibrium. In mammals the labyrinth is composed of a system of epithelial spaces and tubes, the membranous labyrinth, within a protective shell of bone, the bony labyrinth. The membranous labyrinth is filled with endolymph and contains the sensory cells. The surrounding spaces outside the membranous labyrinth are filled with perilymph. The labyrinth transduces mechanical stimuli, such as sound pressure waves and movements of the head, into electrical neural responses. Both auditory and rotatory vestibular responses arise from inner ear fluid movement.

Although the endolymph and perilymph both are extra-cellular fluids, they differ widely in composition. Endolymph resembles an intracellular fluid, with potassium as the predominant cation, low sodium and a high chloride concentration (Sterkers et al. 1988). An ionic concentration gradient in the endolymphatic compartment is present along the cochlea from the base to the apex; a decrease of the potassium and chloride concentration and an increase of the sodium concentration (Salt and Thalmann 1988). Endolymph is positively polarized with respect to perilymph. This endolymphatic potential declines from the base to the apex of the cochlea. The specific chemical composition of endolymph and the generation of the transepithelial positive potential are considered to be regulated by the membrane-bound sodium-potassium activated adenosine triphosphatase (Na/K-ATPase) in the marginal cells of the stria vascularis, as well as in the dark cells of the utricle and the cristae ampullares of the semicircular canals (Kuijpers et al. 1969). Perilymph has a chemical composition resembling that of a plasma ultra-filtrate with a low potassium concentration and sodium as the predominant cation (Sterkers et al. 1988). Also the composition of perilymph throughout the cochlea is not homogeneous, the concentrations of several ions, amino acids, glucose and proteins differ between the scala vestibuli and the scala tympani (Salt and Thalmann 1988). Inner ear functions depend on both inner ear fluid homeostasis and inner ear pressure. Although inner ear pressure is constantly changing, these pressure fluctuations do not affect the mechano-electrical transduction of both auditory and vestibular responses in the normal ear. In the normal ear, no hydrostatic pressure gradient exists between the

endo- and perilymphatic compartments. Hydrostatic pressure of the inner ear is determined by multiple variables, especially the production and absorption of inner ear fluids, cerebrospinal fluid pressure, middle ear pressure and the elastic properties of the membranous labyrinth (Andrews et al. 1991; Böhmer 1993). Disturbances of the inner ear fluid pressure have been regarded by numerous authors as an underlying cause of inner ear disorders like tinnitus, vertigo and fluctuating hearing loss (Sismanis 1987; Böhmer 1991, 1993). Rapid changes in ambient pressure and the application of high-level infrasound are known to produce different forms of inner ear dysfunction, probably by inducing a change in the inner ear hydrodynamics (Densert et al. 1986). Also in longstanding endolymphatic hydrops, when the volume of the endolymph has increased, resulting in a shift of Reissner's membrane into the scala vestibuli, a disregulation of inner ear pressure is believed to exist.

The regulation of inner ear pressure is a complex facet in the physiology of the labyrinth and is not fully understood until now. The inner ear is connected with the cerebrospinal fluid compartment through several pressure transfer routes. The endolymphatic compartment is indirectly connected with the subarachnoid space by the endolymphatic duct and sac. The perilymphatic compartment is connected with the subarachnoid space by the cochlear aqueduct, Hyrtl's fissure, perivascular and perineural routes. Most important pressure transduction canal is the cochlear aqueduct, which runs from scala tympani near the round window membrane to the subarachnoid space at the posterior cranial fossa (Carlborg et al. 1982, 1992; Toriya et al. 1991). Inner ear pressure changes can be corrected by means of fluid flow through the cochlear aqueduct (Beentjes 1972; Marchbanks and Reid 1990; Carlborg et al. 1992; Konradsson et al. 1997). To study the process of inner ear pressure regulation, most ideally pressure is measured within the inner ear. Such an invasive measurement technique is not suitable for human ears. In 1964 Wiederhielm developed the servo-controlled micropipette system to measure pressure in small compartments (Wiederhielm et al. 1964). In 1975 this method was applied for the first time to measure inner ear fluid pressure (Angelborg and Ågerup 1975). The micropipette of the servo-controlled micropipette system is filled with a 2 molar sodium chloride solution, and is connected with a pressure transducer (Figure 1). The tip of the micropipette is inserted into an inner ear fluid compartment, which contains fluid with a lower molarity. Simultaneously the electrical potential at the micropipette tip can be

measured, to ensure proper placement of the pipette-tip. The electrical resistance is measured between the solution in the micropipette and the fluid compartment under investigation (recorded by a reference electrode in the soft tissue surrounding the cochlea). As a result of the pressure (changes) within the inner ear compartment, the interface between both fluids within the pipette-tip shifts, which changes the electrical resistance of the loop between the inner ear compartment with the low molar solution and the micropipette with the high molar solution. The resistance, however, is kept constant by a feedback loop to a pressure pump, which changes the pressure inside the micropipette to keep the interface between both fluids in the tip at a constant level. The pressure generated by the pump can easily be recorded with a conventional pressure transducer (Böhmer 1993).

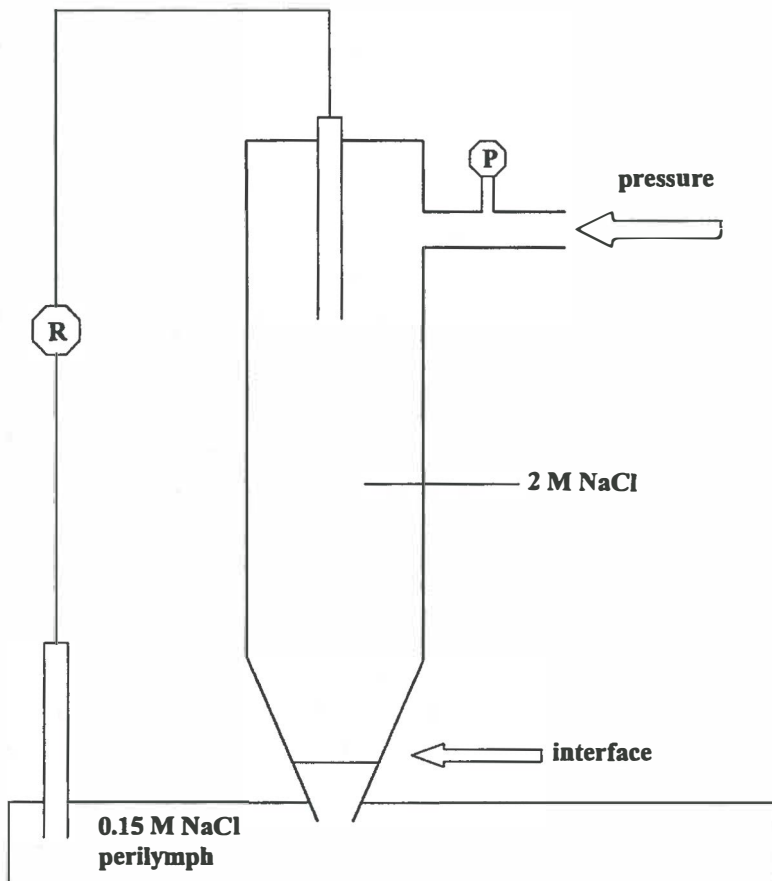


Figure 1 - Principle of the servo-controlled micropipette system

Objectives of this study

The aim of this study is to improve our basic knowledge concerning the dynamics of inner ear pressure regulation. Improved knowledge of the inner ear physiology might help in better understanding the pathophysiology of patients suffering from audiovestibular symptoms caused by inner ear pressure dysregulation.

In guinea pigs inner ear pressure was measured invasively in the scala tympani or the scala media, during various ways of inner ear pressure manipulation. Pressure in the scala tympani and the scala media was measured using a micropipette connected with a servo-controlled micropipette system (WPI 900A micro-pressure system). The dynamics of the inner ear pressure changes were related to the functional aspects of the cochlear aqueduct. Morphological aspects of the cochlear aqueduct were investigated by a light microscopic study, with emphasis on the ductus perioticus and its connection with the round window membrane at the scala tympani.

The anatomy of the inner ear and the physiology of the inner ear fluids, endolymph and perilymph, is reviewed in *Chapter 2*. Production, composition, interrelationship and the regulation of hydrostatic pressure of both inner ear fluids are discussed. Special emphasis is given to the scientific state of the art regarding the cochlear aqueduct and its relation to the inner ear, both morphological and functional.

In *Chapter 3*, the results are described of inner ear pressure measurement in the scala media during infusion or withdrawal of (artificial) endolymph into the same compartment. Time constants for the process of inner ear pressure equalization during pressure increase and decrease were calculated from fits, made to the pressure equalization curves.

Chapter 4 contains the results of perilymphatic pressure measurements during square wave-wise and pulse-wise manipulation of the pressure in the cerebrospinal fluid compartment. Time constants for the inner ear pressure equalization were calculated from fits, made to the pressure equalization curves.

In *Chapter 5*, inner ear pressure is measured during square wave-wise pressure manipulation successively in the cerebrospinal fluid compartment and the external ear canal, in the same guinea pig. Time constants for the process of inner ear pressure equalization were calculated and compared, for the different ways of inner ear pressure manipulation.

In *Chapter 6*, the flow resistance of the cochlear aqueduct is measured during continuous infusion of artificial perilymph into the scala tympani, or withdrawal of perilymph from the scala tympani, during various inner ear pressures in the same guinea pig.

Chapter 7 contains the results of a light microscopic study of the cochlear aqueduct, with emphasis on the opening of the cochlear aqueduct towards the scala tympani, and the connection of the periotic duct with the round window membrane. These morphological findings are related to the results of the functional experiments as described in the earlier chapters. The possible function of the cochlear aqueduct in the complicated process of inner ear pressure regulation is discussed.

In the *final Chapter* the results of this study are summarized and discussed.

Chapter 2

ANATOMY AND PHYSIOLOGY OF THE INNER EAR FLUID SYSTEM

I. Anatomy of the inner ear

The bony and membranous labyrinth

The inner ear or labyrinth contains the important sense organs for hearing and the maintenance of equilibrium. In mammals the labyrinth – literally, a ‘structure of winding passages’ – consists of a joined series of membranous sacs and ducts (the otic or membranous labyrinth) within a protective shell of bone (the periotic or bony labyrinth), situated in the petrous portion of the temporal bone. The bony labyrinth is the toughest part of the skull. The membranous labyrinth is filled with endolymph, a fluid with an ionic composition resembling intracellular fluid. The surrounding spaces outside the membranous labyrinth are filled with perilymph, a fluid of extracellular ionic composition that resembles a plasma ultrafiltrate. The components of the bony labyrinth are the cochlea, the vestibule and the semicircular lateral (horizontal), anterior and posterior canals. The membranous labyrinth consists of the ductus cochlearis (scala media) in the bony cochlea; the endolymphatic sac and duct in the bony aqueduct of the vestibule, the utricle and saccule within the bony vestibule; and three membranous semicircular ducts, within their corresponding canals. The anatomy of the bony and membranous labyrinth is illustrated in Figure 1.

The cochlea is divided into three chambers: scala tympani (ST), scala media (SM) and scala vestibuli (SV). In humans these three chambers form a spiral of approximately 2.5 turns around the central modiolus. In guinea pigs this ascending spiral consists of 3.5 turns. Inside the ductus cochlearis is the organ of Corti, in which the mechanical vibrations of audible sound are transduced into neural electrical responses, which provide vertebrates with the opportunity to hear. The organ of Corti, originally described by Corti in 1851, resides on top of the basilar membrane throughout the length of the membranous cochlea. This structure is composed of sensory cells, supporting cells and nerve fibers. There are two types of sensory cells with different characteristics: the inner and outer hair cells. The hair bundles of the inner hair cells are arranged in one row and are slightly U-shaped, with their apices directed towards the outer hair cells. The tops of the hairs (stereocilia) are located in Hensen’s stripe. The hair bundles of the outer hair cells are arranged in three rows in the form of a wide triple W at the basis, with their

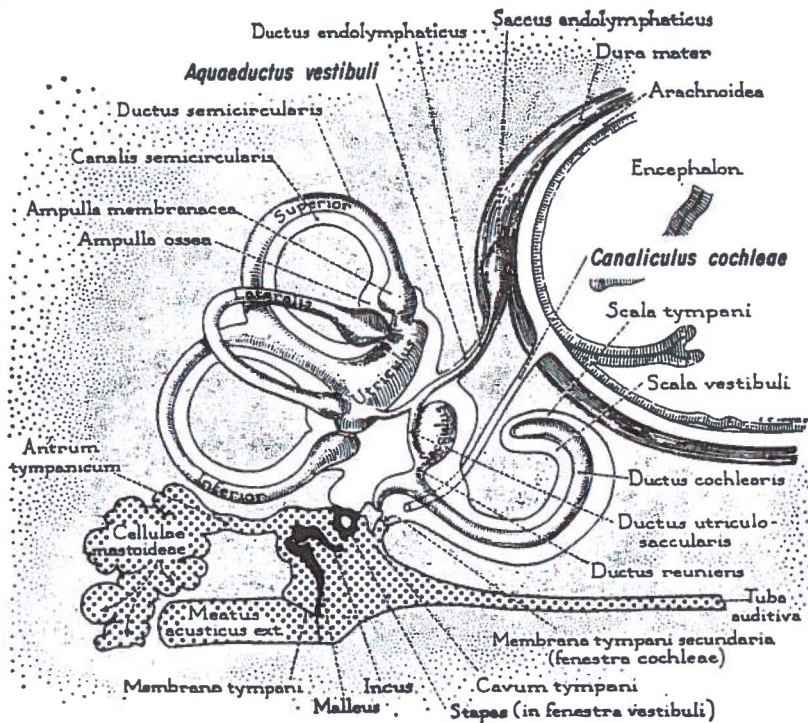


Figure 1 - Anatomy of the bony and membranous labyrinth and structures of the middle ear. The cochlear aqueduct (Canaliculus cochleae) runs from the perilymphatic compartment near the round window membrane (Membrana tympani secundaria) to the cerebrospinal fluid compartment.

apices pointing in the same direction as those of the inner hair cells. The stereocilia of the outer hair cells are firmly attached to the tectorial membrane. The stereocilia are in contact with endolymph, whereas the basolateral membranes of the hair cells are in contact with fluid of a perilymph-like composition. The distribution of cochlear fluids appears to be a major factor in the extreme sensitivity of the cochlea as a mechanoelectric transducer. The framework supporting the organ of Corti is provided primarily by the inner and outer pillar cells and Deiter's cells. Deiter's cells, or outer phalangeal cells, support the basis of the outer hair cells. The cell bodies of the outer hair cells are surrounded by Nuel's space, which is filled with Corti (peri)lymph. Adjacent to the Deiter's cells are Hensen's cells, next to which are the Claudius cells (Kimura 1984). The cell bodies of the inner hair cells are surrounded by inner phalangeal cells, inner pillar cells and border cells (Figure 2).

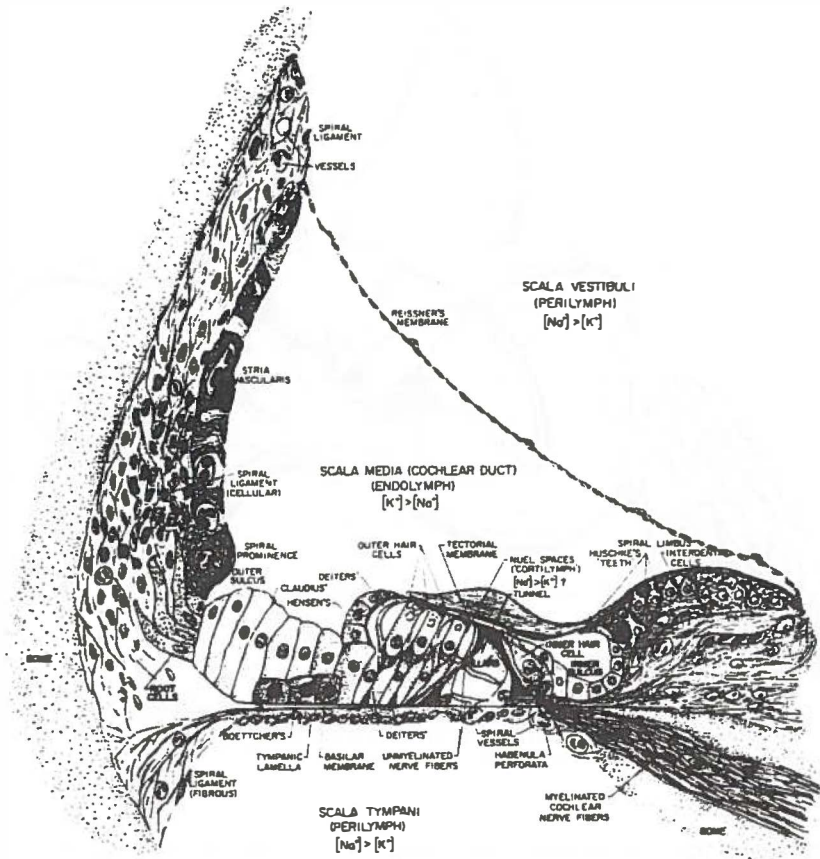


Figure 2 - Sketch of a cross section of the cochlear duct. In scala media the organ of Corti is situated, with haircells, pillar cells, Deiter's cells, Hensen's cells and Claudius cells.

In contrast with the cochlea, the sensory epithelium of the vestibular labyrinth is distributed over five localized spots: in the maculae of the utricle and the saccule and in the three ampullae of the semicircular ducts. The utricular macula lies in a horizontal plane, at right angles with the macula of the saccule, which lies in a vertical plane (in man, with the head in the upright position). The maculae contain sensory cells, with a single kinocilium and many stereocilia, and supporting cells. Both sensory and supporting cells are overlaid with the flattened otoconial membrane, in which numerous crystals, or otoconia, are embedded. The utricle is the larger of the two vestibular sacs, and has apart from the utriculosaccular duct five openings which are the utricular ends of the semicircular ducts (Figure 3). The saccule is anteriorly connected with SM of the cochlea by the ductus reunians; the endolymphatic

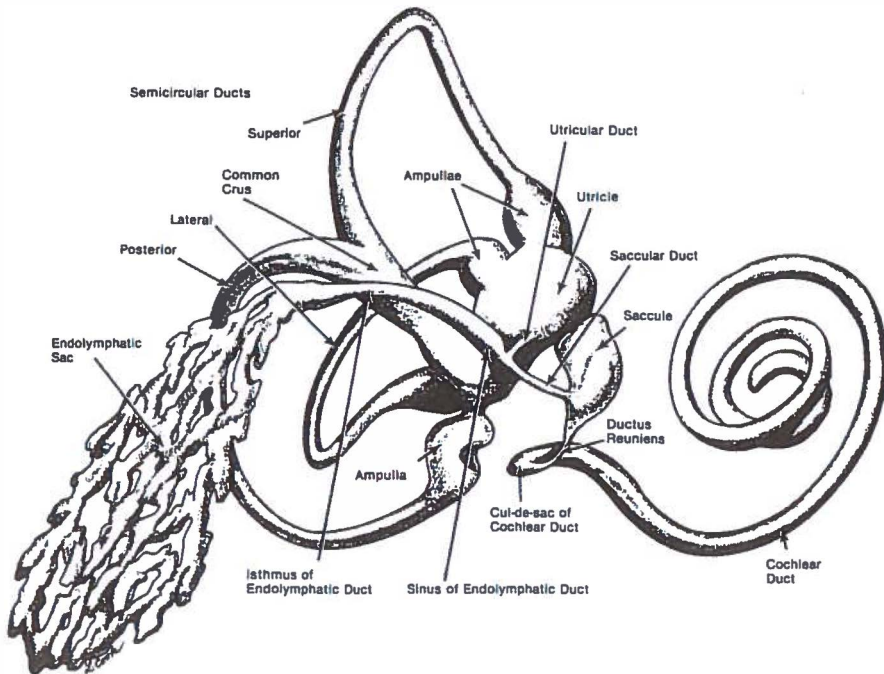


Figure 3 - Sketch of the endolymph filled membranous labyrinth, as viewed from medially. The utricular and saccular ducts join to form the sinus of the endolymphatic duct. The saccule is connected with scala media of the cochlea by the ductus reunions.

duct arises from its posterior part, where it is joined by the utriculosaccular duct. The sensory cells of the semicircular ducts are located in their expanded (ampullary) ends, on top of the cristae.

The sensory epithelium of the cristae is not flat but oppositely curved in two perpendicular directions and is best described as saddle shaped. This epithelium is also formed by sensory and supporting cells, like in the organ of Corti. In the ampullary cristae a wedge-shaped cupula covers the hair bundles. It is a fibrogelatinous structure that forms a fluid-tight connection between the roof of the membranous ampulla and the sensory epithelium. The sensory cells of the maculae and the ampullae have the same structure (with one kinocilium and many stereocilia on their free surfaces) and comprise type I and type II cells. Type I cells are flask-shaped and type II cells are elongated. The location of the kinocilium defines the polarity of the sensory cells, which governs the changes that occur in vestibular neural cells when the ciliary bundle is deflected.

The cochlear fluid compartments

The middle chamber of the cochlea, SM, contains endolymph, whereas the outer chambers, ST and SV, are filled with perilymph. The volumes of cochlear perilymph and endolymph are relatively small. In the guinea pig these volumes are 16 μ l and 2 μ l respectively (Salt and Thalmann 1988). In cross section, the SM forms a triangular compartment between the ST and SV, as illustrated in Figure 4.

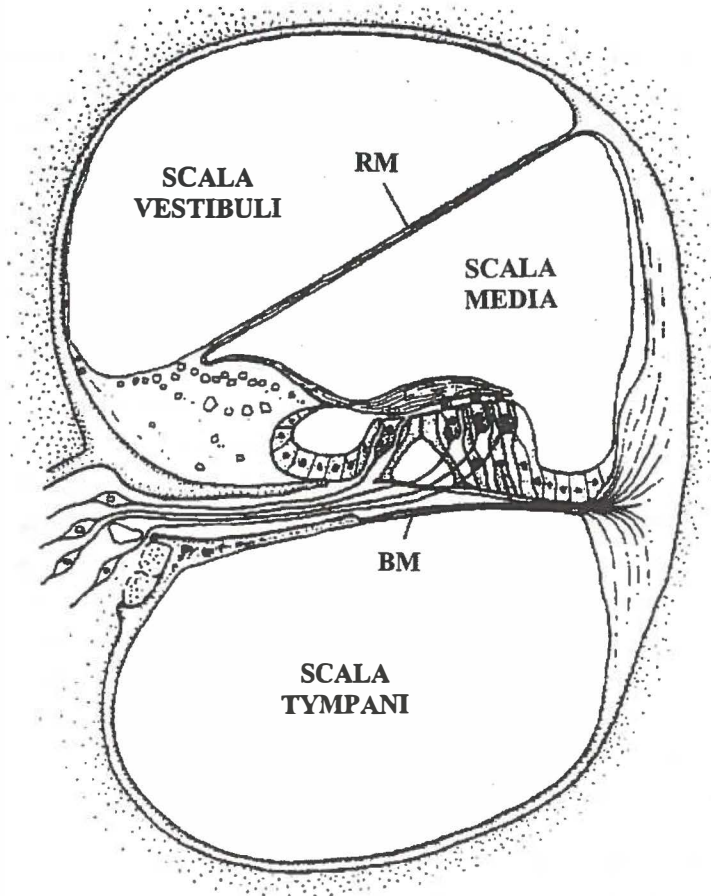


Figure 4 - Cross section of scala tympani, scala media and scala vestibuli. RM = Reissner's membrane, BM = Basilar membrane.

ST and SV communicate through the helicotrema at the apex of the cochlea. In addition, both compartments are connected with other fluid-filled spaces as shown in Figure 1. The basal turn of ST is connected with the cerebrospinal fluid compartment in the fossa posterior by the cochlear aqueduct. The basal turn of SV is connected with the perilymphatic space of the vestibule and the basal turn of SM is connected with the saccule by the ductus reuniens. Thus, all three cochlear scalae have ducts, allowing communication with other fluid-filled spaces.

Both ST and SV have fenestrae to the tympanic cavity: the round window (fenestra cochleae) and the oval window (fenestra vestibuli) respectively. The round window (RW) is closed by the secondary tympanic membrane, the round window membrane. It lies in a deep niche covered by the overhanging promontory. The niche is triangular in shape. The average length of its walls in humans is: anterior 1.5 mm, superior 1.3 mm, posterior 1.6 mm. The RW membrane is not flat but curves towards the scala tympani of the basal coil of the cochlea. Viewed from the middle ear it is concave. In humans the shape of the membrane varies from round to spatulate, with average longest and shortest diameters of 2.30 mm and 1.87 mm respectively (Wright 1995). The membrane consists of three layers: an outer mucosal layer, a middle fibrous layer containing capillaries, nerves, fibroblasts, collections of collagen, elastic fibres and often melanocytes. The inner layer is a continuation of the cell layer lining the scala tympani, consisting of flat mesothelial cells with wide intercellular spaces. The oval window is a nearly kidney shaped opening that connects the tympanic cavity with the vestibule. It is closed by the base of the stapes and its surrounding annular ligament. The long axis of the fenestra is horizontal, with the head in the upright position, and the slightly concave border is located inferior. The size varies; on average it is 3.25 mm long, and 1.75 mm wide in humans (Wright 1995).

SM is separated from the SV by Reissner's membrane. The basilar membrane, which separates SM from the ST, runs in radial direction from the osseous spiral lamina to the spiral ligament. Attached to the upper surface of the basilar membrane lies the organ of Corti. Its surface is covered by the tectorial membrane, which extends from the spiral limbus to the outermost row of Deiter's cells or to the Hensen's cells. The outer wall of the cochlear duct is formed by the stria vascularis and the spiral prominence, covering the

spiral ligament. SM is completely bounded by tissues so that there is no direct fluid connection between endolymph and perilymph. The cells surrounding the endolymphatic compartment constitute an endolymph/perilymph barrier, characterized by tight intercellular junctions (zonulae occludentes) to limit paracellular solute movement. Reissner's membrane was first described by Reissner in his thesis "De auris internae formatione" in 1851. The membrane is avascular, and consists of two different cell layers. The endolymphatic side of the membrane is made up of squamous epithelial cells joined by tight junctions. The epithelial cells display numerous microvilli in the endolymphatic surface. A thin basement membrane separates these cells from the upper perilymphatic layer: mesenchymal cells without tight junctions. Reissner's membrane is attached to the superior part of the spiral limbus and extends to the lateral cochlear wall, where the epithelial cell layer becomes continuous with the stria vascularis. The stria vascularis is a highly vascular, multilayer epithelium that forms the lateral wall of the scala media. The intra-epithelial blood vessels in the stria vascularis lie parallel to one another following the direction of the cochlear turns. The stria vascularis is presumed to be involved in the formation of endolymph, the supply of endolymphatic oxygen and the maintenance of the endocochlear potential. The tissue architecture is shaped by three layers of cells, the marginal, intermediate and basal cells, and blood vessels. The marginal cells border the endolymphatic space and the basal cells connect the stria vascularis to the spiral ligament. The marginal cells (dark cells) are of ectodermal origin, whereas the intermediate and basal cells (light cells) are of mesenchymal descent (Anniko and Nordemar 1980). The marginal cells, like the epithelial cells of Reissner's membrane, have numerous microvilli on the endolymphatic surface. The cells of the basal layer connect the stria vascularis to the spiral ligament and constitute both an anatomical and functional barrier in the compartmentalization of the scala media. The marginal and intermediate cells are in close contact with the cells of Reissner's membrane at its insertion at the stria vascularis. There is a gradual transition between the cells of the stria vascularis and the cells of Reissner's membrane.

The endolymphatic duct and sac

The endolymphatic duct and sac in guinea pigs were first described by Cotugno in 1761, and got their present names in 1873 from Hasse. A first

detailed description of their morphology was given by Guild in 1927, while the ultra-structure of the endolymphatic sac in man was first described by Schindler only in 1980. The endolymphatic duct extends from the junction of the saccular and utricular ducts at the medial wall of the vestibule: the diverging part (*the sinus*). The duct narrows at its entrance into the vestibular aqueduct (*the isthmus*) and it ends halfway down the vestibular aqueduct, where it dilates to form the flat funnel-shaped endolymphatic sac (Schuknecht and Gulya 1986). The epithelium of the endolymphatic duct is of a simple squamous or low cuboidal type and it rests on a basal lamina. The cell contacts are loose, with few tight junctions (leaky epithelium). The luminal surface of the duct possesses a few short microvilli projecting into the endolymph. There are small thin-walled capillaries close to the epithelium.

The endolymphatic sac can be described as being divided into three parts. The *proximal portion*, which is the first dilatation of the sac, is entirely located inside the vestibular aqueduct. The *intermediate or rugose portion* lies partly inside and partly outside the vestibular aqueduct in most experimental animals, whereas it lies entirely within the bony vestibular aqueduct in man. The *distal portion* is flat and lies in close contact with the sigmoid sinus in the dural fold (Wright 1995). The proximal part of the sac exhibits a change in its epithelial structure compared with that of the duct with the appearance of a more cuboidal type of cell. The apical part of the cell membrane exhibits more microvilli in the sac than in the duct. Also in the sac there are more tight junctions between the cells and the subepithelial tissue is more vascularized than in the duct. The epithelial lining of the intermediate part consists of cylindrical cells, irregularly arranged in protruding papillae and deep crypts. With the electron microscope two types of cells can be distinguished: light cells and dark cells. Both cell types are rich in mitochondria. The light cells have more microvilli at their apical surfaces than the dark cells. Neighbouring cell membranes are connected by several tight junctions, thus defining the epithelium as tight. The complex folds of the epithelial lining may give the impression that there is no lumen. However, leucocytes, cellular debris and macrophages may be found in the narrow fluid channels between the epithelial cells. The areolar connective tissue has a rich capillary network in close proximity to the epithelial lining. Close to the side of the sac are thin-walled lymph vessels surrounded by fibroblasts and connective tissue. The epithelial lining of the distal portion is cuboidal, except at its extremity, where it

is squamous. The cell surface exhibits fewer microvilli. The epithelium is apparently tight and non-leaky because of several intercellular sealing strands.

The cochlear aqueduct

The cochlear aqueduct connects the scala tympani with the subarachnoidal space at the posterior cranial fossa. Further digression on this structure will be given in the fourth paragraph of this chapter.

II. The inner ear fluids

Cortilymph and subtektorial lymph can be found in the cochlea besides endolymph and perilymph. The intralabyrinthine fluids include three different functions. The mechanical function is the transmission of the movement of the stapes to the organ of Corti. The electrochemical function of the endolymph in the cochlea is to provide an electrolyte gradient to create a difference in voltage across the sensory cells in the organ of Corti. The biochemical function of the perilymph of the cochlea is to provide metabolic support for the organ of Corti by appropriate concentrations of energy-rich substances, such as glucose, lactate-pyruvate and aminoacids.

Composition of perilymph

The ionic composition of perilymph is similar to that of other extra-cellular fluids in which Na^+ is the predominant cation. The K^+ content of SV perilymph is typically found to be approximately twice that for ST perilymph. Also the concentrations of glucose, several amino acids and proteins in SV are higher than in ST, whereas the Na^+ content is somewhat lower in SV compared to ST. It is therefore incorrect to assume that perilymph composition is homogeneous throughout the cochlea, as different locations show small but systematic differences. Although perilymph has similarities with plasma, there are differences in composition. Perilymph is not identical to a plasma ultrafiltrate. The osmolarity of perilymph does not differ much from that of blood plasma. In guinea pigs, ST osmolarity is 292.9 mOsm/kg H_2O , while that of SV perilymph is 293.5 mOsm/kg H_2O . (The osmolarity of plasma is averaged to be 239.5 mOsm/kg H_2O). Table 1 summarizes the typical ionic content

found for perilymph in ST, SV, SM and for CSF as found in the guinea pig (Salt and Thalmann 1988).

	ST Perilymph	SV Perilymph	Endolymph	CSF
Na ⁺ (mM)	147	141	1	145
K ⁺ (mM)	3.4	6.7	158	2.7
Ca ²⁺ (mM)	0.68	0.64	0.023	
Mg ²⁺ (mM)			0.011	
pH	7.28	7.26	7.37	7.28
Cl ⁻ (mM)	129	130	136	7.28
HCO ₃ ⁻ (mM)	19	18	21	19
Osmolarity (mOsm/kg H ₂ O)	293	294	304	
Electrical potential (mV)	0	5	70-90	0

TABLE 1 - Cochlear fluids composition. ST=scala tympani, SV=scala vestibuli, CSF=cerebrospinal fluid.

Composition of endolymph

The ionic composition of endolymph is unlike any other extra-cellular fluid in the body. The predominant cation in endolymph is K⁺, whereas the Na⁺ content is exceptionally low. The endolymph is also characterized by a high concentration of Cl⁻. There are small but significant differences in ionic concentrations in endolymph between the cochlea and the vestibule. The endolymph in the vestibule has a lower concentration of K⁺ and a higher concentration of Na⁺, Ca²⁺ and Mg²⁺. An ionic concentration gradient is present along the cochlea; from the base to the apex a decrease for K⁺ and Cl⁻ concentrations and an increase for the Na⁺ concentration. The endolymph compartment is electrically polarized with respect to perilymph and plasma: the endocochlear potential. This endolymphatic potential of +70 to +90 mV declines from the base to the apex of the cochlea. Endolymph osmolarity is significantly higher than that of perilymph and plasma. It declines from the base to the apex of the cochlea, following the K⁺ and Cl⁻ gradients (Salt and Thalmann 1988). Endolymph osmolarity in the basal turn of the cochlea was found to be 304.2 mOsm/kg H₂O in the guinea pig (see Table 1).

Perilymph dynamics

Perilymph in ST and SV is thought to be of different origin. Several experiments have indicated that the CSF is important for the formation of perilymph of ST, whereas plasma is the main precursor of perilymph of SV (Scheibe and Haupt 1985; Thalmann et al. 1982). Perilymph derived from CSF enters the cochlea through the cochlear aqueduct. Tracers (radiolabeled sucrose and mannitol) injected into the cerebrospinal fluid compartment have been found in ST and very little in SV (Sterkers et al. 1987). After blockage of the cochlear aqueduct the composition of the perilymph of ST was modified, whereas the composition of the perilymph of SV was not changed (Bergman et al. 1979). However, surgical obstruction of the cochlear aqueduct alone does not induce pathological changes in the cochlea, which suggests that the duct alone is not essential for the maintenance of perilymph composition (Kimura et al. 1974).

The perilymph of SV is produced locally in the cochlea as an ultrafiltrate of blood. After intravenous administration of radiolabeled mannitol and sucrose, these molecules appeared earlier and reached higher concentrations in SV than in ST. The protein content of perilymph is lower than that of plasma, but higher than that of the cerebrospinal fluid compartment. If the composition of blood is changed experimentally, the changes are reflected more rapidly in the perilymph than in the cerebrospinal fluid. The blood-perilymph barrier across which the ultrafiltration takes place is formed by the endothelial cells of the perilymphatic capillaries.

Capillaries have been described in the SV portion of the spiral ligament close to the attachment of Reissner's membrane that could be the site of ultrafiltration. This mechanism involves a perilymph flow depending on the site of resorption. However, the flow rates in ST and SV are so low that their influence is negligible, because passive diffusion occurs at a much higher rate than volume flow (Salt and Thalmann 1988). A third source of perilymph is epithelial secretion, especially for perilymph of the SV. Possible alternative mechanisms include passive diffusion, facilitated transport, or active transport of solutes. Exchange is presumed to occur across a blood/labyrinth barrier, comprised of the pericytes, fibrocytes, and endothelial cells associated with the capillaries of the spiral ligament.

Endolymph dynamics

The high concentration of K^+ and the low concentration of Na^+ in endolymph are maintained by Na/K pumps in the marginal cells of the stria vascularis and in the dark cells of the utricle and the ampullae. The maintenance of the endolymphatic potential (EP) and high endolymphatic K^+ requires an active ion transport mechanism. The stria vascularis has been demonstrated to have an extremely high metabolic rate. It has a high concentration of the membrane-bound Na-K-adenosine-tri-phosphatase (Na/K-ATPase), adenylate cyclase and carbonic anhydrase, which are enzymes associated with the active pumping of ions and transport of fluid into the endolymph. Potassium is actively transported into the marginal cells by the action of Na/K-ATPase in the basolateral membranes. Potassium transport into the SM is then facilitated by ion channels in the apical membranes of the marginal cells. The adenosine-tri-phosphatase is an enzyme with three-high affinity sites for binding Na^+ ions at the cytoplasmic surface. Two high-affinity binding sites for K^+ ions as well as a binding site for the cardiac glycoside ouabain are present at the outer surface of the membrane. During transport three Na^+ ions are moved out and two K^+ ions are moved into the cell per hydrolysed ATP molecule (Kuijpers et al. 1969). The EP originates from an active secretion of K^+ into the endolymph, achieved by an electrogenic transport of K^+ into endolymph (using Na/K-ATPase). The endolymphatic transepithelial potential in the semicircular canals and in the utricle is independent of the endocochlear potential. Apart from the marginal cells of the stria vascularis Na/K-ATPase is also identified in the dark cells of the utricle and cristae ampullaris of the semicircular canals. The activity of the Na/K-ATPase decreases proportional to the endolymphatic potential from the base to the apex of the cochlea. The K^+ secreted into the endolymph originates from perilymph rather than blood plasma. The main origin of endolymph is perilymph. Studies have shown that after perilymphatic perfusion with radioactive K^+ and Cl^- , these tracers appeared rapidly into the endolymph. After intravenous administration of the same tracers penetration in endolymph was much slower.

There is no consensus in the literature how the endolymph homeostasis is maintained. Three mechanisms are described: longitudinal flow, radial flow and local maintenance of homeostasis. Guild (1927) proposed the existence

of endolymph flow: he suggested that endolymph is secreted in the cochlea (produced by the marginal cells of the stria vascularis and the dark cells of the utricle and the ampullae) and flows through the ductus reuniens, the saccule and the endolymphatic duct, to be resorbed by the endolymphatic sac. The difference in osmolarity between endolymph and perilymph was suggested as a possible driving force for bulk flow. Salt et al. (1986, 1988) stated that the mean longitudinal flow rate in the cochlea is far too low to account for the known endolymphatic turnover. However, during acute disturbances of endolymph volume (in experimental situations) an apically or basally directed flow in the cochlea plays a significant role in the correction of this sudden volume disturbance (in guinea pigs) (Salt and DeMott 1995, 1997).

An alternative hypothesis that of radial endolymph flow was proposed by Naftalin and Harrison (1958). They suggested that endolymph was secreted by Reissner's membrane and resorbed by the stria vascularis.

It is also possible that endolymph homeostasis may be maintained without a concomitant fluid flow. As in the normal state longitudinal flow cannot be a significant homeostatic mechanism, endolymph is probably not secreted in volume. Endolymph homeostasis can be assumed to be dominated by local ion transport processes. The ionic composition could be maintained by active ion transport processes in which the total amount of solute into or out of the endolymph would balance the amount of solute leaking passively across the membranous boundaries. If water equilibrates passively, then endolymph composition would be maintained without volume flow necessarily taking place.

It is also possible that all three of the mechanisms described above coexist, but vary in their contribution to endolymph volume and composition under different conditions. It must be emphasized that volume flow is not the only mechanism by which solutes in the cochlea can reach the endolymphatic sac. Other mechanisms include passive diffusion (if a concentration gradient exists) and, for charged solutes, electrophoresis under the influence of the difference in electric potential between the cochlea and the saccule. Thus the regulation of endolymph volume and the regulation of endolymph composition are probably accomplished by different regions of the ear.

The endolymphatic sac is believed to play a role in the regulation of endolymph volume (Salt and DeMott 1997). Following dissection of the endolymphatic sac, a hydrops (=larger than normal volume) can occur (Kimura and Schuknecht 1965). The dark cells in the endolymphatic sac (chief cells) have a potential endocrine function: they contain organelles that can synthesize, secrete and absorb proteins. The light cells in the sac contain many mitochondria. The endolymphatic sac also contains immunocompetent cells, such as lymphocytes and macrophages, implicating a function in local immunodefence. The sac is also believed to have a function in the degradation and absorption of otoconia.

III. Hydrostatic pressure of the inner ear

Maintenance of the hydrostatic pressure of the inner ear fluids is important for a proper functioning of the labyrinth. Both auditory and rotatory vestibular responses arise from inner ear fluid movement. Sound is a variation of pressure that is at least two orders of magnitude smaller than the steady state pressure maintained within the inner ear. Inner ear pressure is determined by multiple variables of which the most relevant are: the production and absorption of inner ear fluids, the cerebrospinal fluid pressure and the pressure in the middle ear cavity. The external environment influences the pressure within the middle ear, which is transmitted to the inner ear through the oval and round windows. The elastic properties of the membranous labyrinth also influence perilymphatic pressure, and, to a greater extent, endolymph pressure. Various other variables that affect inner ear pressure are blood pressure, respiration, coughing, sneezing, heartbeat, posture, stress, concentration of aldosterone in the blood and oxygen saturation. Blood pressure can influence inner ear pressure directly via the inner ear vascular tissue and indirectly via the cerebrospinal fluid, which is affected by blood pressure as well. Although inner ear pressure is constantly changing, these changes do not disturb auditory or vestibular stimuli.

In normal ears the endolymphatic pressure is equal to the perilymphatic pressure. The endolymphatic and perilymphatic compartment of the cochlea are separated by the highly compliant Reissner's membrane and the basilar membrane, which has a lower compliance that changes from base to apex. Although basic for understanding normal inner ear function, our knowledge of

intralabyrinthine fluid and pressure dynamics is only fundamental and remains largely theoretical. The reason for this is that direct measurement of inner ear pressure in the different compartments is complicated in animals and impossible in humans. Increased knowledge on how the above mentioned factors affect the intra-labyrinthine pressure is important for our understanding of for instance the pathophysiological mechanisms of perilymphatic fistulae, alternobaric vertigo and endolymphatic hydrops.

Inner ear fluids

The intrinsic pressure within the endolymphatic and perilymphatic compartments is generated through the difference in formation and absorption of endo- and perilymph. Endolymph is mainly produced by the marginal cells of the stria vascularis and the dark cells of the utricle and cristae ampullaris of the semicircular canals. Absorption of endolymph mainly takes place in the endolymphatic sac. A surgical obliteration of the endolymphatic sac and duct in guinea pigs leads to an endolymphatic hydrops (Kimura and Schuknecht 1965). These animals develop a fluctuating hearing loss (Horner 1993). This obliteration model for endolymphatic hydrops has been used extensively in the research concerning Menière's disease. In experimentally induced endolymphatic hydrops the endolymph volume increases. The resulting shift of Reissner's membrane towards the scala vestibuli also affects the volume of perilymph. In early stages of hydrops, distension of Reissner's membrane does not result in an endolymphatic pressure change. In longstanding hydrops, the compliance of Reissner's membrane decreases with the progressive distention. In this condition, a pressure gradient between the endolymphatic and perilymphatic compartment can develop (Böhmer 1993).

An increase of hydrostatic pressure in the perilymphatic compartment can correct itself by means of a fluid flow through the cochlear aqueduct towards the cerebrospinal fluid compartment. In patients with either an unusual wide communication between the cerebrospinal fluid compartment and perilymph-filled spaces, or transmission of a pathologically raised intracranial pressure to the perilymph, the fluid shift is mainly in the opposite direction (Mangabeira-Albernaz et al. 1992). These patients may suffer from a *perilymphatic hypertension*, a term which is under discussion. It is misleading to conclude that "hypertension" is an increase in absolute perilymphatic pressure alone

(Marchbanks and Reid 1990). On the contrary, the intracranial and intracochlear (perilymphatic) pressure parallel each other (Marchbanks 1990). Two prominent anatomical deficits are described to be responsible for the abnormal wide communication between the perilymphatic space and cerebrospinal fluid compartment: a particularly wide cochlear aqueduct, and a wide internal auditory canal or a fundus defect (Schuknecht and Reisser 1988). In patients with these abnormalities the substantial pulsation of the brain and inside the cerebrospinal fluid compartment can be transferred directly to the perilymph-filled spaces of the cochlea (Densert et al. 1981). Besides that, normal pressure hydrocephalus, benign intracranial hypertension (pseudotumor cerebri) or any increase in intracranial pressure have a major impact on cochleovestibular function by the resulting increase of the perilymphatic pressure (Sismanis et al. 1987, 1990). Although the underlying disorders are completely different, the clinical similarities are surprising. Main clinical symptoms in these patients are sensorineural hearing loss, tinnitus and vertigo, which can have a Menière-like character.

Cerebrospinal fluid pressure

The intracranial pressure changes on a second to second basis. Superimposed on the baseline intracranial pressure are periodic changes due to cardiovascular activity and respiration. Over longer periods, there is a diurnal rhythm and a monthly cycle in females. However, these daily pressure variations do not affect the functioning of the normal inner ear.

The influence of CSF pressure on the inner ear pressure depends on the patency of the pressure transfer routes between the CSF compartment and the labyrinth, i.e. the cochlear aqueduct primarily and the endolymphatic duct and sac and theoretically the perivascular- and perineural spaces, secondarily (Carlborg et al. 1982, 1983; Kishimoto et al. 1983; Densert et al. 1986). The cochlear aqueduct is the most important pressure transduction canal (by means of fluid transport between the scala tympani of the inner ear and the cerebrospinal fluid compartment). Carlborg and Farmer (1983) increased the CSF pressure in cats by means of fluid infusion into the subarachnoid space, both in animals with the cochlear aqueduct patent and in animals with the cochlear aqueduct blocked. In the animals with an open cochlear aqueduct the rise of pressure in the cerebrospinal fluid compartment and in the

perilymphatic compartment was simultaneously (there was a time delay of less than a second) and identical. In the animals with the cochlear aqueduct blocked, some increase of the perilymphatic pressure was recorded, but it occurred much slower and was smaller than the change of the CSF pressure. This slow and small pressure transfer probably takes place by indirect pressure transmission via the endolymphatic sac and duct.

The contribution of the perineural and perivascular routes is not fully understood; it has been suggested that these channels may mediate a direct communication between the CSF compartment and the perilymphatic compartment. When the pressure transfer in the experiments mentioned above would have been mediated through direct and fully open communication routes, the perilymphatic pressure should have reached the same levels as the cerebrospinal fluid pressure.

The influence of hydrocephalus and intracranial hypertension on hearing has been studied extensively (Tandon et al. 1973; Sismanis 1987, 1998). In some of these studies the tympanic membrane displacement analyser (Marchbanks 1984) was used as a tool for measuring the perilymphatic pressure in humans (Bohndorf and Ernst 1997; Ernst and Lenarz 1993). In patients with an internal hydrocephalus that had been treated by a ventriculoperitoneal shunt for years, symptoms of tinnitus, hearing loss or vertigo could occur, following a shunt blockade resulting in an increased intracochlear pressure. Replacement of the shunt resulted in normalisation of the intracranial and intracochlear pressure, and complete relief of complaints (Bohndorf et al. 1997; Moss et al. 1989, 1990). In patients with intracranial hypertension (CSF pressure >200 mm of water) Sismanis reported symptoms of pulsatile tinnitus, hearing loss, dizziness or vertigo, and aural fullness. Especially the pulsatile tinnitus and hearing loss could be relieved by lowering the intracranial pressure by means of a lumbar puncture or a ventriculoperitoneal shunt (Sismanis 1998).

The tympanic membrane displacement analyser also was used for non-invasive perilymphatic pressure measurements in patients with Menière's disease (Rosingh et al. 1998). Rosingh studied the relation between CSF pressure and perilymphatic pressure by measuring the effect of postural changes on perilymphatic pressure in patients suffering from Menière's

disease. If the cochlear aqueduct patency in these patients would deviate from the patency in healthy humans, a postural change might affect inner ear pressure differently. He found no significant deviations from the results of the same experiments in healthy humans.

Büki et al. (2002) detected intracranial and intracochlear pressure changes by measuring otoacoustic emissions, in gerbils. De Kleine et al. (2001) studied stimulus frequency- and click evoked otoacoustic emissions in normal hearing subjects in the upright and supine position. Both types of otoacoustic emissions were influenced by posture changes, indicating a change of the intracochlear fluid pressure.

In patients exposed to either spinal anaesthesia, surgery for acoustic neuroma or other neurosurgery, hearing can also reversibly be affected. The degree of hearing loss and the number of frequencies involved increases with an increasing amount of CSF loss (Walsted et al. 1991, 1994). Change in the cochlear fluid volumes after CSF aspiration, with a transitory reduction in the perilymphatic volume and resultant endolymphatic expansion, is suggested to be responsible for these observed findings (Walsted 1998).

Middle ear pressure and the compliance of the window membranes

The labyrinth is exposed to ambient pressure changes, mediated by the middle ear (Konradsson et al. 1997). If the inner ear is considered to be a rigid fluid filled space, it is connected with the CSF compartment by means of the bony cochlear aqueduct and it is separated from the tympanic cavity by the round and oval window membranes. Inner ear pressure is influenced by the sum of middle ear pressure and the pressure that the cochlear windows exert on the inner ear fluids. The latter pressure depends on the compliance of the windows. In practice this means that the risk of inner ear damage will be increased by any process that increases the compliance of the cochlear windows, and by extremes of middle ear pressure, brought about by Eustachian tube dysfunction combined with barometric pressure changes.

For the treatment of Menière's disease Densert et al. (1997) have developed a device to influence inner ear pressure, by manipulation of the middle ear pressure: the Meniett®. With the Meniett® a complex pressure wave can be applied to the external ear canal. This pressure will be transmitted to the

middle ear via a ventilation tube. The pressure change is then transmitted to the inner ear fluids, mainly through the round window membrane. Since the inner ear fluids are not compressible and the membranous labyrinth is enclosed in a bony capsule, an increase in perilymphatic pressure may force an excess of endolymph out of the endolymphatic compartment through release routes, such as the endolymphatic duct and sac, thus activating longitudinal flow, resulting in a decrease in endolymphatic fluid. Densert et al. report a positive effect of treatment with the Meniett® (Densert et al. 1997; Ödkvist et al. 2000), but the clinical effect of the Meniett® is still subject of further evaluation.

IV. Perilymphatic communication routes

The most important connecting pathway between the perilymphatic and intracranial fluids is the cochlear aqueduct (perilymphatic duct). The cochlear aqueduct runs from scala tympani of the basal cochlear turn, near the round window membrane, through the petrous part of the temporal bone to the subarachnoid space at the posterior cranial fossa. Its position is just below the internal auditory meatus and just above the ninth nerve. Parallel to the cochlear aqueduct runs Hyrtl's fissure (the tympanomeningeal fissure) from the area inferior to the round window to the fossa posterior (Eggston and Wolff 1947). Also parallel to the cochlear aqueduct run 2 accessory canals. The first one contains the inferior cochlear vein and exits the scala tympani adjacent to the cochlear aqueduct. The vein empties into either the petrosal sinus or the jugular bulb. This bony canal is eponymically referred to as the canal of Cotugno, who originally described it in 1760. Less frequently occurring is the second one with a vein from the tympanic cavity which eventually joins the canal of Cotugno (Palva 1970). Other suggested routes are the perineural spaces connected with the perilymphatic part of the labyrinth.

The cochlear aqueduct

The cochlear aqueduct (CA) has been studied for 300 years, beginning with its discovery by DuVerney in 1684. Within the lumen of the CA is a loose network of connective tissue, continuous with the arachnoid membrane, often with a central lumen within it (Jackler and Hwang 1993; Gopen et al. 1997). Since 1949 the term 'periotic duct' is used to designate the fluid-containing

tissue within the bony cochlear aqueduct. The periotic duct is characterized by dense and sparse portions (Nishimura et al. 1981). Erythrocytes, leucocytes, phagocytes and lymphocytes were occasionally observed within the periotic duct tissue. At the cranial side the dura mater and the arachnoid run along the bony wall of the aqueduct, entering the lumen in a layered condition. Where the dura enters the lumen, it becomes so thin, that it is indistinguishable from the lining layer of the bony wall. The arachnoid tissue is taken to be continuous with the periotic duct. The periotic duct forms an intricate mesh network within the lumen of the cochlear aqueduct, containing fibroblasts, collagen fibrils and blood vessels. In the main duct the fibroblasts are arranged parallel to the long axis. At the internal orifice, there is no barrier membrane at the opening towards the scala tympani. The periotic duct tissue is relatively dense, and fibroblasts are arranged more perpendicularly, nearer to the demarcation between the internal orifice and the scala tympani. However, the interior of the lumen is nowhere closed. Hence, fluid flow in both directions is possible.

The endolymphatic structures (cochlear duct, semicircular canals, vestibular aqueduct) are derived from the otic vesicle, which is ectodermal in origin. By contrast, the CA forms in the mesoderm, which condenses around the membranous labyrinth and gradually ossifies during embryogenesis. The CA is initially apparent as an outpouching of the subarachnoid space, which consists of loose connective tissue (Streeter 1918). At first it is rather straight, but with progressive otic capsule ossification it elongates and becomes curved. The diameter of the cochlear opening of the periotic duct remains constant with gestational age, whereas the medial opening into the CSF space progressively increases in diameter (Spector et al. 1980).

In the literature a great variety in the diameter and length of the cochlear aqueduct is reported. In most studies it is not made clear at what part of the cochlear aqueduct de diameter was measured. As the diameter of the cochlear aqueduct varies along its course, these findings can be misinterpreted. In some studies ears of patients with an otologic history were investigated. So most of these studies are not reliable. Su et al. (1982) compared mean CA diameters at various ages and determined it to be greatest in infancy, averaging 0.25 mm compared to 0.19 mm in adults. Gopen et al. (1997) demonstrated the contrary. In a study of 101 normal

temporal bones of healthy humans (10 bones per decade of life until the age of 100 years) no systematic changes in patency of the cochlear aqueduct as a function of age were found. Estimates of the length of the human adult CA range from 6 to 12 mm (Ritter and Lawrence 1965; Anson et al. 1965; Palva and Dammert 1969; Rask-Anderson et al. 1977), while the radius of the aqueduct varies from 0.15 to 0.02 mm. The smallest diameter is located 200-300 μm from the cochlear end of the aqueduct and has a mean value of 0.138 ± 0.058 mm (Gopen et al. 1997). In guinea pigs the CA is much shorter than in humans. Ghiz et al. (2001) measured a length of 2 mm. Shinomori et al. (2001) reported, based on 3-D reconstruction of histologic material, that the volume of the CA in the guinea pig was estimated to be 0.113 mm^3 . This is close to the volume observed by Ghiz et al. (2001).

Functions of the CA have been suggested to include:

- (1) *the supply of perilymph from the cerebrospinal fluid.*
- (2) *a protective effect against inflammation between the scala tympani and the CSF.*

Experiments in animals, as well as numerous histologic observations in man, demonstrate that the CA can act as a conduit for spread of infection from the CSF to the inner ear (Yanagita et al. 1984; Bhatt et al. 1991; Schuknecht et al. 1993).

- (3) *a phagocytotic and filtering effect on contaminants.*

Macrophages and erythrocytes have been found in the cochlear aqueduct, not able to pass through. In addition, the observation has been made that red blood cells may traverse the CA to reach the cochlear basal turn after a fatal subarachnoid haemorrhage (Holden and Schuknecht 1968).

- (4) *the transmission and equalization of pressure differences between the perilymph and the CSF (Nishimura et al. 1981)*

The aqueduct appears to filter out pressure pulses in the CSF, and prevents them from affecting cochlear function (Gopen et al. 1997). The cochlear aqueduct acts as a low-pass filter, preventing inner ear damage after large and rapid pressure changes of the cerebrospinal fluid. Transmission of CSF pressure increase to the inner ear compartments appears to be faster than the transmission of CSF pressure decrease. This indicates that the CA may have a larger resistance to fluid flow in one direction than in the other.

Large cochlear aqueduct

Enlargement of the cochlear aqueduct is often mentioned in the otologic literature, usually in its purported association with sensorineural hearing loss, stapes gusher, and transotic cerebrospinal fluid leak (Jackler and Hwang 1993). Although enlargement of the CA is frequently cited in both the clinical and radiographic literature, no criteria have been developed to define the radiographic diagnosis of this proposed anomaly. Jackler and Hwang (1993) studied the diameter of the CA in CT scans of 100 human ears. They divided the CA in four parts: (1) the lateral (internal) orifice into the cochlea, (2) the part traversing the otic capsule, (3) the part traversing the petrous apex, and (4) the medial aperture into the posterior fossa. The part traversing the otic capsule was the narrowest part, and never exceeded 2 mm in diameter in their study. They proposed as criterion for the diagnosis of CA enlargement a CA diameter exceeding 2 mm in its mid-otic capsule portion, on a high resolution CT scan.

Schuknecht and Reisser (1988) have proposed that the perilymphatic ooze, which is encountered in stapes surgery for otosclerosis, is partly due to a widely patent cochlear aqueduct. As the narrowest segment of the cochlear aqueduct has the largest effect on fluid flow, enlargement must involve the otic capsule portion, not merely the medial orifice. Fluid flow resistance of a tube varies with the fourth power of its diameter, so even slight enlargements of the CA may have a substantial impact on perilymphatic flow into scala tympani. Theoretically a minor diameter increase at the narrowest point (for example from 0.02 to 0.04 mm, which decreases flow resistance with a factor 16 significantly increases fluid flow. Such an enlargement is beyond the resolution of CT scanning. Theoretically, also in a normal shaped aqueduct, a deficiency of the membranous mesh that fills the aqueduct may also result in an increased flow through the cochlear aqueduct. However, Gopen et al. (1997) state that even the widest cochlear aqueducts cannot support the perilymphatic flow rates observed in 'gushers'.

Until now, details of the dynamic properties of the CA in equalizing the CSF and inner ear pressure are not fully understood. As a normal inner ear pressure is a prerequisite for normal functioning of the inner ear, increased knowledge of the characteristics of the most important pressure transduction canal for the inner ear can help in the understanding and probably the

treatment of patients suffering from otologic symptoms caused by perilymphatic fistulae, endolymphatic hydrops or intracranial hypertension.

Chapter 3

DYNAMICS OF INNER EAR PRESSURE RELEASE, MEASURED WITH A DOUBLE BARRELED MICROPIPETTE IN THE GUINEA PIG

Wit HP, Thalen EO, Albers FWJ.

Dynamics of inner ear pressure release, measured with a double barreled micropipette in the guinea pig.

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Introduction

Insight in the relation between inner ear fluid volume and pressure is important to understand the hydromechanical consequences of endolymphatic hydrops, which is thought to be the histopathological substrate of Menière's disease (for a review see: Horner, 1993). It has long been thought that endolymphatic hydrops (higher than normal fluid volume) causes endolymphatic hypertension. And in same models the assumption was made that this pertension displaces the basilar membrane away from its normal equilibrium position, giving a mechanical explanation for hearing loss associated with Menière's disease (Tonndorf 1957; Klis and Smoorenburg 1985).

Because a force is needed to displace the elastic basilar membrane from its equilibrium position (Gummer et al. 1981; Milier 1985; Olson and Mountain 1991), permanent displacement does indeed require a hydrostatic pressure difference between endolymph filled scala media and perilymph filled scala tympani. In normal (guinea pig) ears no difference can be measured between perilymphatic and endolymphatic pressure (Yoshida and Lowry 1984; Long and Morizono 1987; Böhmer and Andrews 1989; Takeuchi et al. 1990; Andrews et al. 1991; Böhmer 1991; Schröder 1997). By infusion of artificial endolymph into scala media of the guinea pig Takeuchi et al. (1991) investigated the possibility to create endolymphatic hypertension. Simultaneous recording of perilymphatic and endolymphatic pressures, however, showed no pressure difference during and after infusion. Furthermore both pressures returned to their pre-infusion value within seconds after fluid injection was stopped. So although fluid injection into scala media does create an acute volume increase basalwards of the injection site, even at very low injection rates (Salt and DeMott 1997), it does apparently not create a measurable pressure difference between endolymph and perilymph.

Information on the pressure regulating mechanism of the inner ear may be obtained by studying the recovery process after acute pressure change. Therefore, we injected artificial endolymph into scala media of the guinea pig through one barrel of a double-barrelled micropipette. Through the other barrel fluid pressure and endocochlear potential were measured simultaneously. How volume change affects inner ear pressure depends on

the compliance of the bordering membranes of the inner ear compartment and the flow resistance of pressure release routes. As a first approximation these factors have been incorporated in a very simple model to be described hereafter.

Theoretical model

Pressure release

Firstly we consider the case of pressure release, after having created overpressure in the inner ear by fluid injection. The simplest model for this situation is given in Figure 1, in which the inner ear is depicted by a rigid

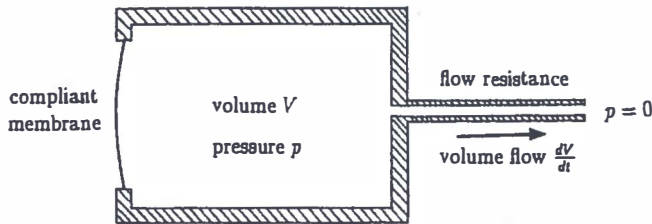


Figure 1 - Model for inner ear pressure release after volume increase. Immediately after volume increase inner ear fluid volume is V and pressure is p . This pressure returns to zero by fluid flow through the tube.

walled cavity, because it is surrounded by bone; except for the windows, which are represented by the compliance C . From the cavity fluid can escape (e.g. through the cochlear aqueduct). Possible parallel escape routes are represented by the flow resistance R . Because no pressure difference has been measured between endolymph and perilymph filled spaces, mechanical properties of membranes between these spaces are neglected in this first approximation. We assume the cavity to be filled with fluid with volume V and pressure p . If p is larger than the outside pressure, fluid will escape through the flow resistance. (For simplicity reasons outside pressure is taken to be equal to zero. This will not affect the generality of the solution: a constant pressure may be added, both to p and the outside pressure.) The simplest case is obtained when volume flow dV/dt is proportional to pressure: $-(dV/dt) = (1/R)p$, and changes of p and V with time are also proportional: $C(dp/dt) = dV/dt$. Combining these two assumptions yields the differential

equation: $-(dp/dt) = \alpha p$, with solution: $p = p_0 e^{-\alpha t}$, in which $\alpha = (RC)^{-1}$. This single exponential function did not fit well to most of our experimental results. Therefore a term was added to the differential equation for pressure, giving: $-(dp/dt) = \alpha p + \beta p^2$. The solution for this equation is: $p = p_0((e^{-\alpha t})/(1 + \gamma(1 - e^{-\alpha t})))$, in which $\gamma = (\beta/\alpha)p_0$. This solution will be referred to as "double exponential function". A special case is obtained for $\alpha = 0$. Then the differential equation turns into: $-(dp/dt) = \beta p^2$, with solution $p = (p_0)/(1 + \beta p_0 t)$, which is no longer an exponential function, but a hyperbola. In the general case volume and volume flow are both functions of p : $V = g(p)$ and $-(dV/dt) = h(p)$. Membrane compliance and flow conductance p (the reciprocal of flow resistance) are then defined as the derivatives of these functions: $C(p) = g'(p)$ and $p(p) = h'(p)$. This gives $-(dp/dt) = h(p)/C(p)$ as the general differential equation for p . Then the consequence of fitting our data with the solution of $-(dp/dt) = \alpha p + \beta p^2$ is that $h(p)/C(p) = \alpha p + \beta p^2$. For the somewhat less general case that $C(p)$ is a constant, this gives a flow conductance $p(p) = h'(p) = \alpha C + 2\beta C p$, which is a linear function of p . And if $p(p)$ is constant, $h(p)$ is proportional to p : $h(p) = (1/R)p$. This results in: $C(p) = 1/(\alpha R + \beta R p)$. Integrating this function gives $V = g(p) = V_0 + (1/\beta R) \ln(1 + (\beta/\alpha)p)$.

Pressure build-up

Next we assume fluid to be injected into the cavity of Figure 1 with a constant flow f , while at $t = 0$ pressure inside and outside the cavity are p_0 . Fluid injection will cause pressure p to rise, and fluid will escape through resistance R (now assumed to be constant for reasons of simplicity) at a rate $f_{out} = p/R$. As long as $f > f_{out}$, the fluid volume in the cavity will increase, according to $dV/dt = f - f_{out} = f - (p/R)$. If we further assume $C(dp/dt) = dV/dt$, in which C is a (constant) compliance, combination with the previous equation will yield the differential equation: $RC(dp/dt) + p = fR$; with solution $p = p_0 + fR(1 - e^{-\alpha t})$; $\alpha = (RC)^{-1}$. So at $t = 0$ pressure starts to rise with a rate $dp/dt = f/C$ until an equilibrium value $p = p_0 + fR$ is reached. In this final situation fluid leaves the cavity with the same flow f as with which it is injected.

Materials and methods

Successful experiments were performed in 8 female pigmented guinea pigs (Harlan Laboratories U.K.; 500-800 g bodyweight), under general anaesthesia

induced by intramuscular administration of ketamine/xylazine (60/3.5 mg/kg). Muscle relaxation was obtained with succinylcholine (2.5 mg/kg). The animals were artificially ventilated through a tracheostoma (Columbus Instruments, model 7950) and body temperature was maintained at 38°C. The animal's head was kept in a stationary position by means of a steel bolt fixed to the skull with dental cement. The bulla was opened by a retroauricular approach and the round window exposed. Through the round window membrane the tip of a double-barrelled micropipette was inserted into scala tympani, and after subsequent perforation of the basilar membrane into scala media. DC potential at the pipette tip was measured to verify its position. Double-barrelled micropipettes were drawn from borosilicate glass (1.5/0.84 mm diameter per barrel) and the tips were bevelled (Narishage EG-40). Tip diameters were around 30 μm per barrel, which is a compromise between low enough flow resistance for fluid injection and tip smallness.

One barrel of the pipette was used to measure inner ear pressure and DC potential (WPI 900A micro-pressure system). Through the other barrel fluid (140 mM KCl + 25 mM KHCO_3 ; Salt and DeMott 1997) could be manually injected into the inner ear with a small steel plunger syringe. The connection between this glass syringe and the micropipette barrel was made of hard-walled teflon tubing, completely filled with fluid. It was verified, by measuring fluid pressure inside the syringe with a miniature solid state pressure transducer (Braun Medical, type MTC P3FU), that fluid flow from the pipette tip returned to zero within 0.1 s after termination of an injection. During and after injection the syringe was kept in a horizontal position at the same level as the animals head. Flow resistance of a barrel was tained by measuring the volume of fluid leaving the pipette tip, when a known pneumatic pressure was applied to the barrel. It was typically $4 \times 10^3 \text{ Pa.s/nl}$. During an experiment inner ear pressure and DC potential values were stored digitally with a rate of 10Hz, after low pass filtering (cut-off frequency 5 Hz). The response time of the micro-pressure system was calibrated to be better than 0.1 s. Fits to relevant portions of the obtained recordings were made off-line with an appropriate software package.

Results

A typical result of an endolymphatic pressure recording is shown in Figure 2.

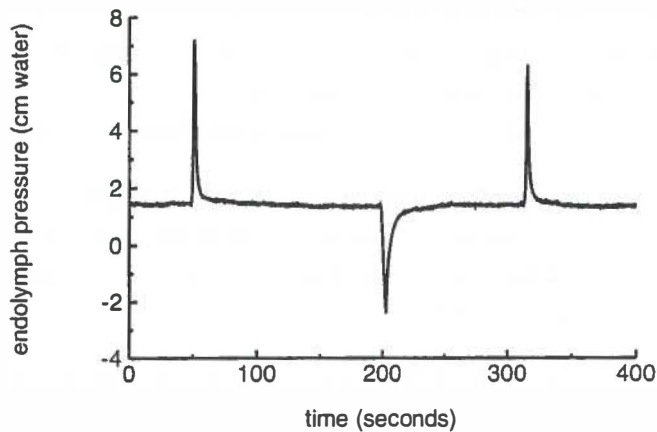


Figure 2 - Temporary endolymphatic pressure increase (or decrease) by injection (or withdrawal) of artificial endolymph through one barrel of the micropipette, followed by returning of pressure to its initial value.

The steady state pressure is about 1.5 cm water. At $t=50$ and $t=310$ seconds fluid is injected during about 2 seconds. In this time approximately $0.1 \mu\text{l}$ of artificial endolymph enters scala media (see Appendix). When injection is instantaneously terminated pressure returns to its initial value, also within seconds. The same happens after fluid withdrawal at $t=200$ s.

Because the endolymphatic potential started to degenerate after more than a few fluid injections, we restricted the number of injections and withdrawals in most animals. In total we analysed the results of 23 injections and 10

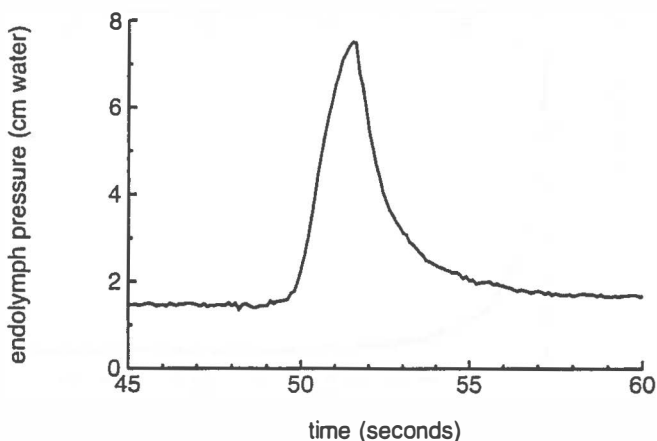


Figure 3 - Detail of figure 2: endolymphatic pressure increase during about 2 seconds, followed by exponential pressure decrease.

withdrawals of fluid. The average maximum pressure change after injections was 4.6 cm water (range 1.1-9.6), and 2.9 cm water (range 0.7-5.8) after withdrawals. The curves, along which pressure returns to its initial value after short over- or underpressure, have an exponential shape (e.g. Figure 3).

Fits with a single exponential curve were, however, only good in 10 out of the 33 analysed recovery curves. In the remaining cases much better fits were obtained with the double exponential function, as given in the theory section. This is shown in Figures 4 and 5.

The results as shown in these figures were consistently found, both for rising and falling curves. For all 33 curves a time constant τ was derived from the fits. This time constant is defined as the time interval in which pressure returns from its maximum value to e^{-1} times this maximum value.

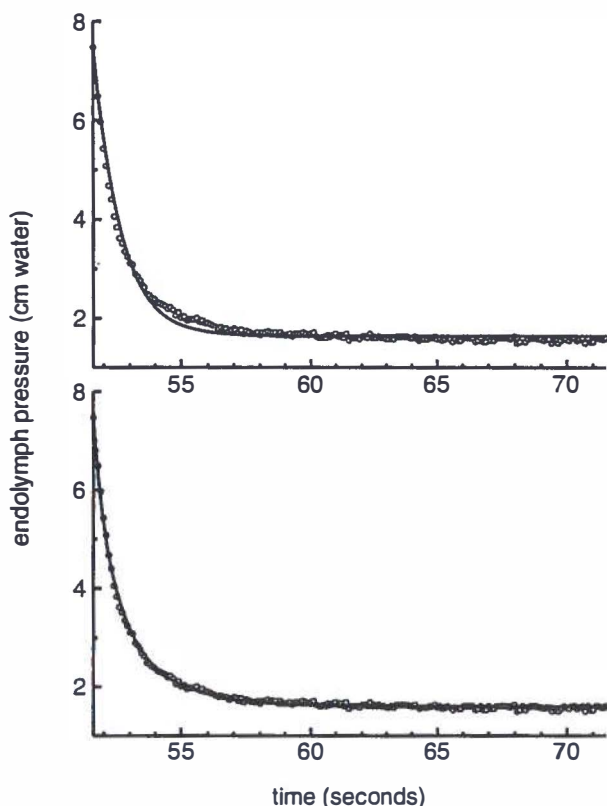


Figure 4 - Shape of pressure return curve after temporary overpressure. Upper panel: fit with single exponential curve. Lower panel: same recording, but fitted with double exponential curve (see Theory Section). Time constant $\tau = 0.99$ s.

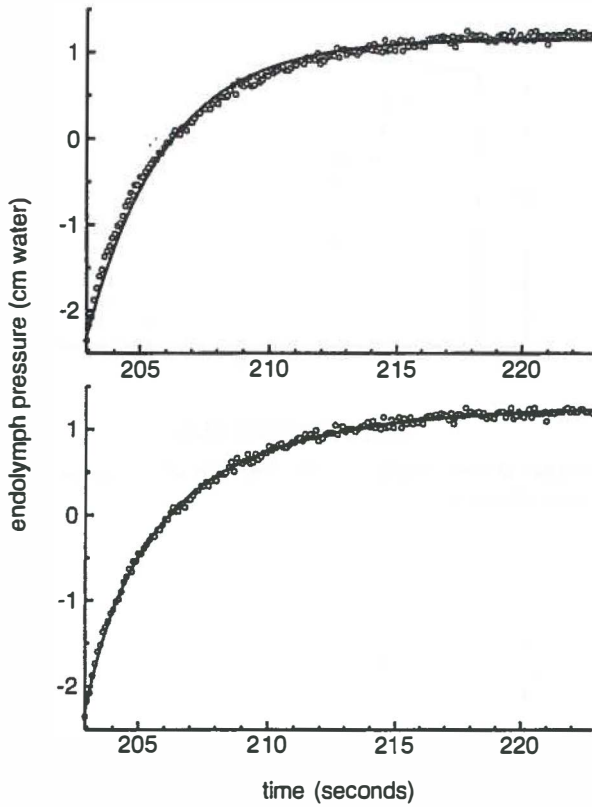


Figure 5 – Shape of pressure return curve after temporary underpressure. Upper panel: fit with single exponential curve. Lower panel: same recording, but fitted with double exponential curve (see Theory Section). Time constant $\tau = 3.03s$.

For the single exponential curve the time constant is simply α^{-1} . For the double exponential curve $\tau = \alpha^{-1} \ln((e+\gamma)/(1+\gamma))$. For recovery after overpressure an average time constant of 1.1 seconds (range 0.5-1.9) was obtained from the fits, and for recovery after underpressure of 2.8 seconds (range 2.1-4.2). Figure 6 gives the distribution for τ for the 33 analysed pressure recovery curves. For ears in which τ was measured both after underpressure and after overpressure the average ratio of both τ -values was 2.6 (range 1.3-3.5). No clear relation can be observed between the values for τ and the maximum pressure change after fluid injection or withdrawal, as can be seen in Figure 7.

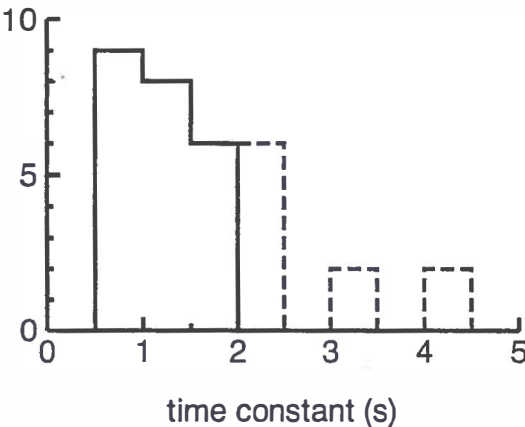


Figure 6 - Distribution of time constants for pressure return after overpressure (solid line) and after underpressure (dashed line).

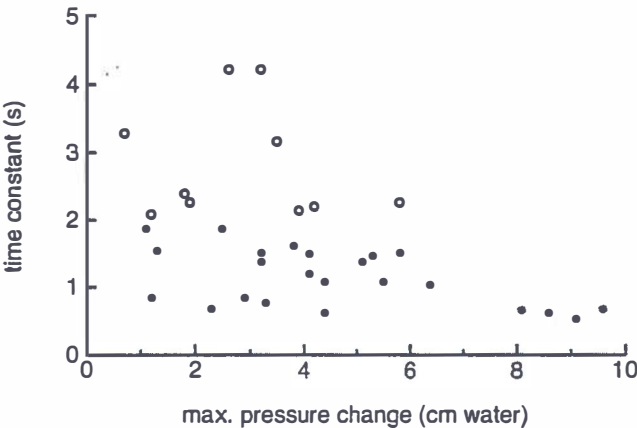


Figure 7 - Relation between time constant for pressure return and (absolute value of) maximum pressure change for temporary overpressure (filled circles) and for temporary underpressure (open circles).

Discussion

To explain the observation that endolymph pressure returns to its initial value after fluid injection into (c.q. withdrawal from) scala media we have to assume that the total volume of fluid in the inner ear has not changed: a permanent increase of the volume of (incompressible) fluid in the inner ear compartments would have made the oval and round window to move outward, increasing the

inner ear pressure by elastic restoring forces. Such a pressure increase is indeed observed during injection, because then fluid goes in faster than it goes out. But in the end the total volume that has been injected in the inner ear has gone out again: the inner ear is a leaky system.

A trivial explanation could be that we did create the leak ourselves, by penetrating scala tympani and subsequently scala media with the micropipette. This did indeed sometimes happen, but in these cases -that were excluded from further analysis- the steady state inner ear pressure was zero instead of a few cm water; which is the normal value for guinea pig inner ear pressure (see Table 1).

Author(s)	Pressure (cm. water)	Number of animals	Method
Feldman et al., 1979	3.21 (\pm 0.34)	21	Servo
Yoshida and Lowry, 1984	4.1 (\pm 0.8)	5	Servo
Long and Morizono, 1987	2.71 (\pm 2.15)	25	WPI
Nakashima et al., 1987	2.5 (\pm 1.0)	9	WPI
Böhmer and Andrews, 1989	2.4 (\pm 0.8)	10	Servo
Andrews et al., 1991	1.20 (\pm 2.94)	10	Servo
Böhmer, 1991	2.1 (\pm 0.6)	10	Servo
Schröder, 1997	2.28 (\pm 1.2)	10	WPI

Table 1 - Inner ear hydrostatic pressure (with standard deviation) in the guinea pig as measured by different authors, either measured with a home-made servo-controlled system ("servo"), or with a commercially available instrument using the same principle ("WPI").

The possibility of fluid escape through the injection pipette can also be discarded because the pipette has a flow resistance that is at least two orders of magnitude larger than the flow resistance through which fluid escapes from the inner ear (see Materials and Methods and Appendix). That pressure normally returns to a steady state value different from zero after fluid injection can be seen in Figure 8.

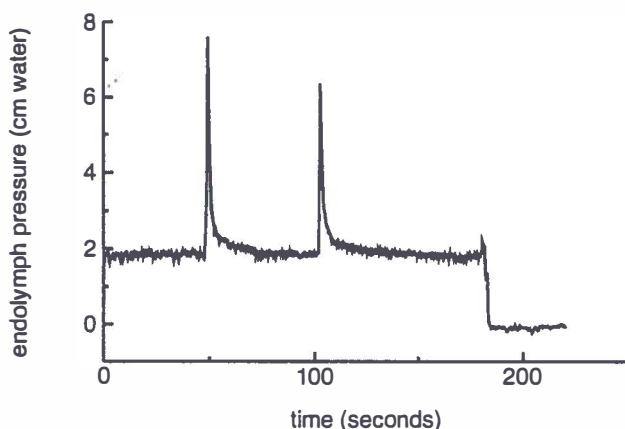


Figure 8 - End of recording. At $t=180$ s the micropipette is withdrawn from the inner ear.

To obtain this figure the micropipette was withdrawn from the inner ear right after the second fluid injection. A pressure fall of almost 2 cm water, when the micropipette leaves scala tympani, is clearly observable. So we must conclude that the normal guinea pig inner ear maintains a fluid pressure of a few cm water, while artificially induced hypertension (or hypotension) disappears within seconds. The same was found by Takeuchi et al. (1991; their Figure 3): During injection of artificial endolymph in scala media of the guinea pig endolymphatic and perilymphatic pressure were raised by approximately 7 cm water, but as soon as injection was terminated these pressures fell back to their pre-infusion value within seconds. The simplest explanation for our observations is that fluid injected into scala media displaces perilymph from the inner ear, and from the results obtained by Carlborg et al. (1982) and Suzuki et al. (1994) it is reasonable to conclude that the cochlear aqueduct is the main escape route for perilymph, which was also proposed by Beentjes (1972) and Takeuchi et al. (1991). In a straightforward experiment, in which a dye was injected into the spinal fluid of living guinea pigs, Jako et al. (1959) showed the open connection between scala tympani and the cerebrospinal fluid spaces: through the round window membrane dye (fluorescein) was seen pouring out of the opening of the cochlear aqueduct within seconds after injection. Further support for the view that perilymph is displaced from the inner ear comes from the fact that the time constant for inner ear pressure release after injection of artificial endolymph, as found in this study (1 to 2 seconds), is the same as that found

for inner ear pressure change after a sudden change of cerebrospinal fluid pressure (Thalen et al. 1997) or a sudden change of ear canal pressure (Thalen 1998) in the guinea pig.

If injected artificial endolymph displaces perilymph, it also has to displace membranous structures that completely (as is assumed) surround the endolymph filled compartments of the inner ear and the vestibular organ. Apparently no extra pressure is needed for such a displacement, because Takeuchi et al. (1991) could not measure a pressure difference between endolymph and perilymph during infusion of artificial endolymph in scala media of the guinea pig. (Accuracy of their measurements was 0.1 mm Hg, while pressure increased in the order of 10 mm Hg (14 cm water or 1.4×10^3 Pa) during infusion). This is remarkable because the maximum volume of injected fluid was 2 μ l, which is as much as the total volume of endolymph in scala media (Salt et al. 1986). As the total volume of endolymph in the inner ear (including the vestibular system) is estimated to be around 5 μ l (Rauch 1964), a sudden increase of this volume by 40 % does apparently not create a pressure difference between the endolymphatic and perilymphatic compartments, measurable with a micro-pressure system, which has an accuracy in the order of 10 Pa. Recently Salt and DeMott (1997b) have confirmed the result obtained by Takeuchi et al. (1991): they also could not measure a pressure difference between endolymph and perilymph, when artificial endolymph was injected. Furthermore, administration of glycerol via a gastrocatheter in guinea pigs (Takeda et al. 1990) decreased perilymphatic and endolymphatic pressures by as much as 8 mm Hg (11 cm water), but again no difference between these two pressures was measurable. Also in the static situation no pressure difference can be measured between endolymph and perilymph in the normal guinea pig ear (Yoshida and Lowry 1984; Long and Morizono 1987; Böhmer and Andrews 1989; Takeuchi et al. 1990; Andrews et al. 1991; Böhmer 1991; Schröder 1997). This could mean that some part of the membranous structures surrounding the endolymphatic compartments has such a high compliance that no measurable pressure difference can exist across it. Böhmer and Andrews (1989) suppose that it is Reissner's membrane that equalizes endo- and perilymphatic pressures, and that Reissner's membrane loses this ability after a longstanding endolymphatic hydrops. Salt and DeMott (1997) on the other hand, based on endolymphatic flow measurements induced by microinjections of artificial

endolymph at low rates, conclude that structures outside the cochlea play a role in the correction of endolymph volume disturbances. If a highly compliant structure, as meant above, can not be found, some other mechanism must be responsible for precise pressure equalization in the inner ear.

That the (vestibular part of) the inner ear is very sensitive to pressure differences between endolymph and perilymph was shown by Zucca et al. (1991). In a preparation of the vestibular system of the frog these authors did already find changes in spontaneous and mechanically evoked nerve activity at a pressure difference as low as 3 Pa (0.3 mm water). Recently stretch activated channels in Reissner's membrane were put forward to play a role in balancing hydrostatic pressure across this membrane (Yeh et al. 1997); but pressures to open these channels were found to be as high as 2×10^3 Pa (15 mm Hg), which is about 10 times the normal hydrostatic inner ear pressure.

Although we did not inject more than a few times a volume of artificial endolymph in the order of 100 μl (see Appendix), which is 5 percent of the total volume of scala media each time, this did already cause a decrease of the endolymphatic potential (EP) in same animals, as illustrated in Figure 9. Our injection rate was about 70 $\mu\text{l/s}$ (see Appendix).

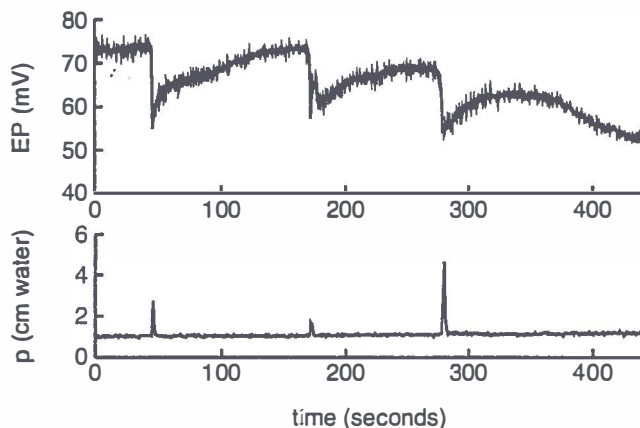


Figure 9 - Degradation of the endolymphatic potential (upper panel) after a few injections of artificial endolymph (lower panel).

A similar observation was made by Takeuchi et al. (1991), who saw an EP decrease during injection of 2 μl of artificial endolymph in the guinea pig, with

a rate of about 14 $\mu\text{l/s}$. But in a later experiment in the same laboratory (Kakigi and Takeda 1998), with a somewhat lower injection rate (8 $\mu\text{l/s}$), a slight increase of EP was seen during and after injection, while no EP change was seen during perfusion (injection with outlet) of artificial endolymph; so it is not the artificial endolymph itself that causes EP changes. What does cause the EP changes is not clear to us at this moment. Kakigi and Takeda (1998) suppose that it could be a direct mechanical effect on the organ of Corti. If this is true, the effect looks to depend critically on the injection rate. A consequence of the fact that EP is changed by fluid injection is that results from such injection experiments should be interpreted with care, as long as it is not known if inner ear pressure depends on EP.

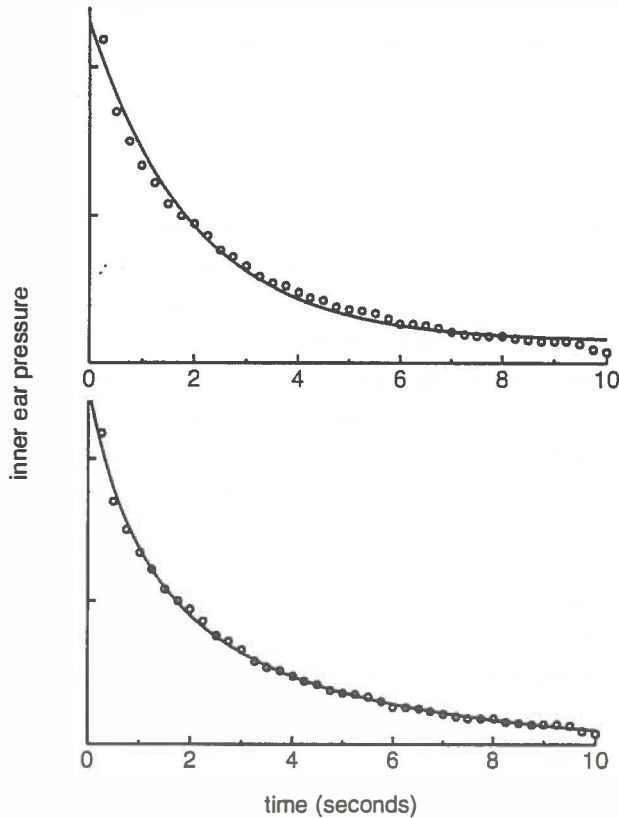


Figure 10 - Inner ear pressure return in the cat after a sudden increase of middle ear pressure. Open circles: recording taken from Carlborg et al. (1982). Solid line: fit with single exponential (upper panel), or with hyperbola (lower panel). Time constant for pressure return $\tau = 2.43$ s.

In order to compare our results for pressure release in guinea pigs, as shown in Figure 4, with results obtained in an other animal we digitized inner ear pressure recordings reported by Carlborg et al. (1982, their Figure 3). These authors studied inner ear fluid pressure change after a sudden increase or decrease of middle ear pressure in the cat. This resulted in an immediate increase or decrease of inner ear pressure, followed by a pressure equalisation within a few seconds. Return of pressure to its initial value was most probably caused by fluid displacement through the cochlear aqueduct, because no pressure release was seen if the aqueduct was blocked. Figure 10 shows the time course of pressure release, as taken from Carlborg et al.'s results. In the upper panel these results are fitted with a single exponential curve. This fit shows systematic differences between the measured and fitted curve, just like in our own results. The best fit was obtained with the hyperbolic curve (see Theory Section), as shown in the lower panel of figure 10. The time constant for pressure release in this figure is 2.4 seconds, somewhat larger than what is found for the guinea pig (see Figure 6).

From the results obtained by Carlborg et al. (1982) it is reasonable to conclude that the cochlear aqueduct is the main route for equalisation of inner ear fluid pressure. If the assumptions that we made in the Theory Section are right, several possibilities exist: If membrane compliance is constant both our results and those from Carlborg et al., as shown in Figures 4, 5 and 10, indicate that the cochlear aqueduct is not a simple flow resistance, like a narrow hard-walled tube would be, but rather behaves as a pressure dependent system for which flow resistance increases with decreasing pressure; possibly caused by the loose network of connective tissue, with which the aqueduct is filled (Svane-Knudsen 1958).

On the other hand Ivarsson and Pedersen (1977) found a round and oval window compliance in human temporal bone preparations that depended on pressure. By taking their data for the round window a perfect fit could be obtained (see figure 11) with $V = V_0 + a \ln(1 + bp)$, as derived in the Theory Section for the case of a constant flow resistance.

The third possibility is that both resistance and compliance are pressure dependent. Anyhow, inner ear pressure release is a non-linear process, which must also be concluded from the fact that the time constant τ depends on flow

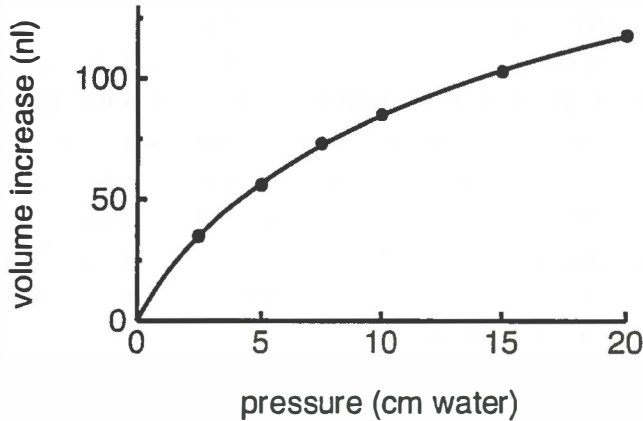


Figure 11 - Filled circles: datapoints for round window volume displacement at different pressures in human temporal bone preparations, as taken from Ivarsson and Pedersen (1977; Figure 4). Solid line: fit with $V = a \ln(1+bp)$, as derived in the Theory Section for the case of a constant flow resistance and a pressure dependent membrane compliance.

direction (see Figures 6 and 7), and further research is needed to unravel which of the above possibilities applies to the inner ear.

The importance of the cochlear aqueduct for pressure release has been questioned because Wlodyka (1978) found a decreasing patency of the aqueduct in man with increasing age. This finding, however, could not be confirmed in a recent extensive study of the anatomy of the human cochlear aqueduct (Gopen et al.1997). Seo et al. (1997) suggest a relation between patency of the human cochlear aqueduct and inner ear symptoms, such as hearing loss and disequilibrium. In a human temporal bone study they find a definitely open aqueduct in 38 out of 43 cases without symptoms, and only in 21 out of 32 cases when inner ear symptoms are present.

Appendix

The injected fluid volumes were so small that we could not measure them directly in our experimental set-up. Therefore they were estimated as follows: The onset slope of the pressure curve in Figure 3 is 5.2×10^2 Pa/s. With $dp/dt = f/C$ at $t=0$ (Theory Section) flow f , which is assumed to be constant during injection, can be found by multiplying this slope by the compliance of the inner ear cavity. It is reasonable to suppose that this compliance is mainly

that of the round window membrane (Ivarsson and Pedersen 1977), which is $0.14 \mu\text{l}/\text{Pa}$ in the guinea pig (Décory et al. 1990), giving a fluid injection rate of $73 \mu\text{l}/\text{s}$. As fluid was injected during 1.7 s , the volume of injected fluid was $124 \mu\text{l}$ in this example. The value for the compliance of the guinea pig round window, as used in the above calculation, is close to that for the human round window; which is $0.2 \mu\text{l}/\text{Pa}$ for small pressure changes (Ivarsson and Pedersen 1977). In first approximation (assuming constant resistance and constant compliance) flow resistance $R = \tau/C$ for pressure release can be estimated to be $7.9 \text{ Pa}\cdot\text{s}/\text{nl}$; using a time constant $\tau = 1.1 \text{ s}$, as found as the average value in our measurements.

Chapter 4

DYNAMICS OF INNER EAR PRESSURE CHANGE CAUSED BY INTRACRANIAL PRESSURE MANIPULATION IN THE GUINEA PIG

*Thalen EO, Wit HP, Segenhout JM, Albers FWJ.
Dynamics of inner ear pressure change caused by intracranial pressure
manipulation in the guinea pig.
Acta Otolaryngol (Stockh) 2001; 121; 470-476*

Introduction

Inner ear fluid volume and pressure regulation are important prerequisites for normal functioning of the inner ear. A better understanding of the hydromechanical interactions between the intracochlear and intracranial fluids, their interdependence and relationship, is essential to our understanding of both normal and pathological physiology of the audiovestibular organ. In patients with Menière's disease or intracranial hypertension symptoms of tinnitus, fluctuating hearing loss and paroxysmal vertigo are thought to be related to an abnormal hydrostatic pressure of the inner ear (Sismanis 1987). Also patients that underwent neurosurgery, involving puncture or drainage of the subdural space, can have temporary hearing loss, tinnitus, fullness and nausea (Walsted et al. 1994).

Intracranial pressure changes are transmitted to the inner ear. In the guinea pig the main route for pressure transfer is the cochlear aqueduct (Beentjes 1972; Carlborg et al. 1992; Kerth and Allen 1963; Marchbanks and Reid 1990). Other routes are the perivascular and perineural spaces, and (indirectly) the endolymphatic sac and duct (Carlborg et al. 1983, 1992). Both in man and guinea pigs the cochlear aqueduct extends from near the round window membrane in the basal turn of scala tympani to the subarachnoid space. Inside this canal a loose network of connective tissue, contiguous with the arachnoid membrane can be found that, although permeable to fluid, limits the patency of the cochlear aqueduct (Jackler and Hwang 1993). The canal is not completely permeable, as macrophages and erythrocytes have been found in it, having been unable to pass through. To date, quantitative details of the dynamic properties of the cochlear aqueduct in equalizing inner ear pressure are scarce. More insight in the pressure regulation mechanism of the inner ear may be obtained by studying the dynamics of inner ear pressure recovery after pressure manipulation. Therefore, in this study intracranial pressure in guinea pigs was manipulated, and pressure changes in the inner ear recorded. The results were described with a simple physical model. Two related intracranial pressure manipulation profiles were studied: square waves and short rectangular pulses.

Materials and methods

Animal preparation

Fourteen female albino guinea pigs (Harlan Laboratories U.K.), having normal pinna reflexes, were used in the experiments. Animals weighed between 650 and 800 grams. The animals were anesthetized with ketamine/xylazine (60 mg/kg / 3.5 mg/kg) and immobilized with succinylcholine (2.5 mg/kg), administered intramuscularly. Tracheotomy was performed and ventilation maintained by respirator (Columbus Instruments, model 7950). Body temperature was kept stable at approximately 38°C using an isothermic blanket. Each animal was placed in the supine position, with the head kept stationary by means of a steel bolt fixed to the skull with dental cement. Heart rate was monitored throughout the experiment. The tympanic bulla of the guinea pig was opened by a retro-auricular approach, and the round window membrane was exposed. The care and use of the guinea pigs in this study were approved by the Animal Studies Committee of Groningen University, protocol number 1173 / 0896-0998.

Intracranial pressure manipulation and measurement

In order to measure and manipulate intracranial pressure, two holes were drilled in the cranium, without causing damage to the dura. In these holes, 1.5 mm diameter short stainless steel tubes were fixed with dental cement. A 1 mm diameter miniature solid state pressure transducer (MTC P3FU catheter type HD 43002, B. Braun, Melsungen) was inserted into one tube, and an infusion system filled with heparinised Ringer's solution was connected to the other tube. In the first five experiments, intracranial pressure was manipulated pulse-wise by means of an electromagnetically driven infusion system. In later experiments, the system was improved and intracranial pressure was manipulated pulse- and square wave-wise by means of an electronically controlled pneumatic system connected to the same infusion system filled with heparinised Ringer's solution. Pulse-wise manipulation of intracranial pressure was performed with a standard pulse width (1 sec) and a standard repetition rate (0.05 Hz) and different amplitudes. Low frequency square wave manipulation of intracranial pressure was performed with square pressure steps having a standard frequency (0.01 Hz) and fixed amplitude. Both for the short pulses and for the square waves rise and fall times for intracranial pressure change were in the order of 0.1 seconds.

Perilymphatic pressure measurement

Hydrostatic pressure in the perilymphatic compartment of the inner ear was measured through a micropipette using a WPI model 900A, servo-nulling micro pressure system. Micropipettes were drawn from borosilicate glass and the tips were bevelled to a diameter of approximately 5µm using a Narishage EG-40 pipette grinder. The micropipettes were filled with 2 M NaCl. By means of a micromanipulator, the micropipette was inserted through the round window membrane into scala tympani where the perilymphatic pressure was recorded. The DC-potential, recorded simultaneously through the same pipette, monitored localization of the pipette tip. An indifferent silver/silver chloride wire electrode was placed in a neck muscle of the guinea pig. Pressure calibration was performed at the beginning of each experiment by lowering the pipette in saline over a known distance.

Data handling

During pressure manipulation, hydrostatic pressure in both the peridural space and the perilymphatic compartment of the inner ear were simultaneously monitored. These pressures and the DC-potential of scala tympani were also stored on computer hard disk with a rate of 10 Hz, after low-pass filtering (cut-off frequency 5 Hz). Responses to repeated stimulus presentations were averaged off-line. The signal component due to the animal's breathing was removed from the response by smoothing its Fourier transform at the frequency of breathing. Least squares fits to the obtained averaged recordings were made with an appropriate software package.

Results

Successful measurements were performed in 14 ears of 14 guinea pigs. In 5 animals intracranial pressure was manipulated with a square wave profile only, and in 4 animals only with short rectangular pulses. In 3 animals intracranial pressure was manipulated both with short pulses, and after that with a square wave profile. In one animal intracranial pressure was manipulated with short pulses and after that with a sinusoidal pressure change profile.

Before intracranial pressure manipulation the mean static pressure measured in scala tympani was 3.95 cm water (range 0.6-6.0). No attempt was made to measure absolute values of intracranial pressure; only the pressure changes

caused by pulse or square wave manipulation were recorded.

Square wave intracranial pressure manipulation

The effect of intracranial pressure changes with a square wave profile was studied in 8 ears of 8 animals. During infusion or withdrawal of artificial intracranial fluid, a step-wise increase or decrease of cerebrospinal fluid pressure was induced. Inner ear pressure immediately followed manipulated intracranial pressure (Figure 1).

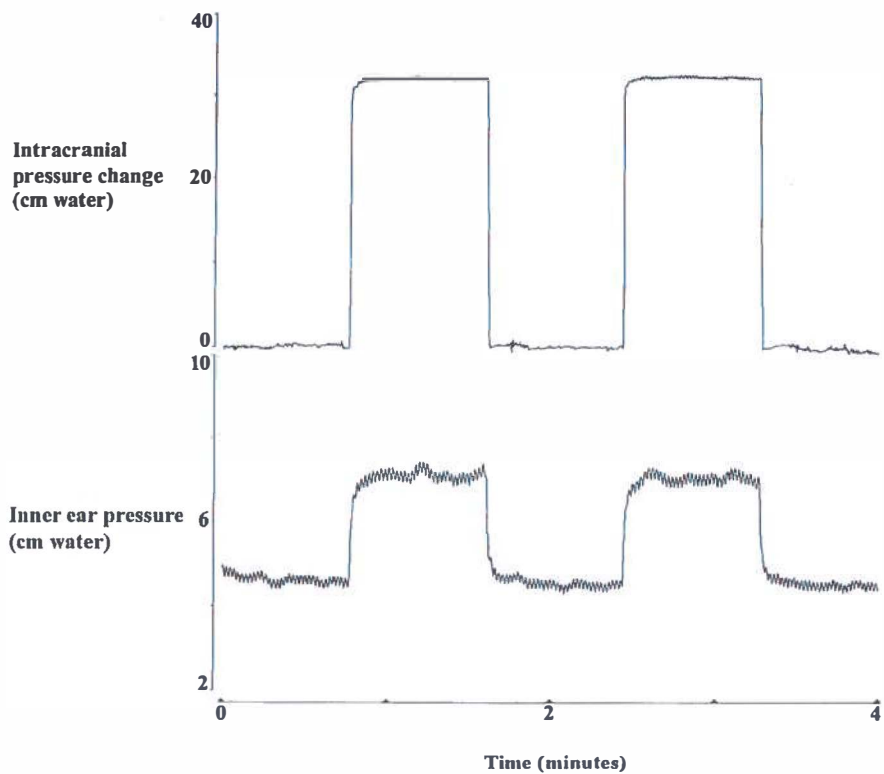


Figure 1- Part of the results of pressure recordings in one animal: changes of inner ear pressure, following square wave-wise manipulated intracranial pressure changes. The pressure equalization process of the inner ear behaves as a low-pass filter.

There was no measurable time lag in the onset of pressure change in the inner ear, compared to the onset of pressure change in the intracranial compartment. The inner ear pressure curve has the shape of a low-pass filtered square wave. The mean change of inner ear pressure was 1.5 cm water (range 0.6-3.3), after a

mean pressure step of intracranial pressure of 29.8 cm water (range 21.2-36.5). The curves along which inner ear pressure stabilized after an intracranial pressure step have an exponential shape. In first approximation, these pressure equalization curves could be fitted with a simple exponential function (Figure 2a), assuming that pressure change with time (dp/dt) is proportional to pressure p . There is, however, a systematic difference between the measured and fitted

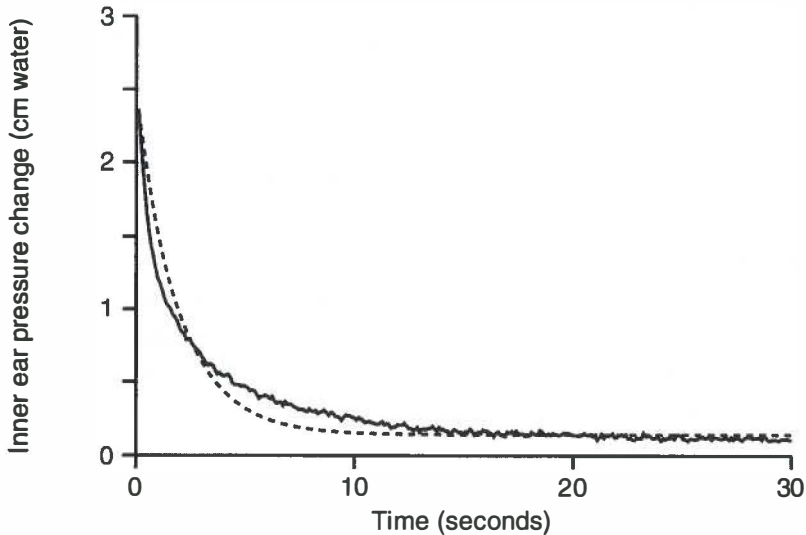


Figure 2a - Fit with a simple exponential function (dashed line) of the inner ear pressure equalization curve following a step-like decrease of the intracranial pressure: fit is not satisfactory.

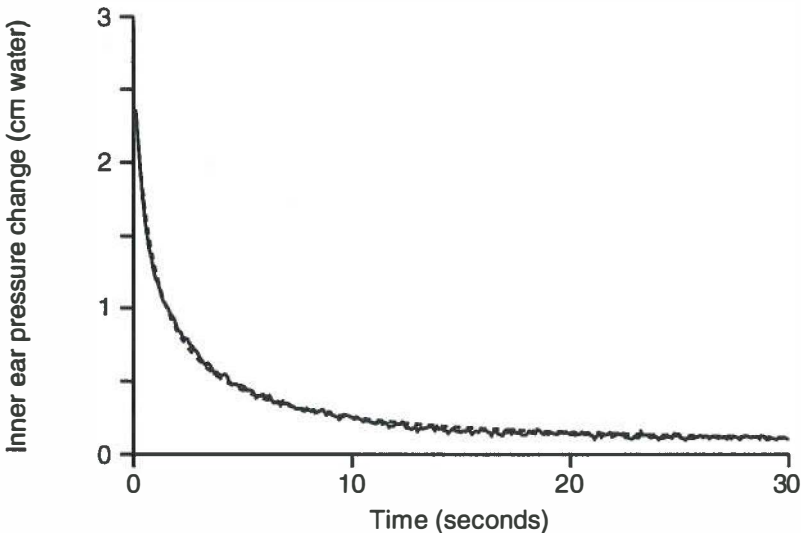


Figure 2b - Fit with a double exponential function (dashed line) of the inner ear pressure equalization curve following a step-like decrease of the intracranial pressure: fit is (almost) perfect.

curves, which was a consistent observation for both rising and falling of inner ear pressure. To obtain a better fit it was assumed that dp/dt is a nonlinear function of pressure, which means that the compliance C of the inner ear bordering structures and/or the flow resistance of the cochlear aqueduct R are not constant. More specifically it was assumed that $dp/dt=ap+bp^2$, in which a and b are constants (Wit et al. 1999). The solution of this equation yields a “double exponential” function, which provided an almost perfect fit to the pressure equalization curves of the inner ear in all measurements (Figure 2b).

For all 16 averaged pressure curves, a time constant (τ) was derived from the fits with an accuracy of better than 0.1 second. The mean time constant obtained was 2.8 seconds (range 1.0-3.6) after pressure increase, and 2.0 seconds (range 0.5-3.4) after pressure decrease. All time constants found (from fits with the double exponential function), for both rising and falling of inner ear pressure, were in the range of 0.5 to 3.6 seconds. Time constants for pressure decrease were smaller than those for pressure increase, in the same guinea pig. This was observed in 8 out of 8 investigated ears. The smaller the time constant, the faster pressure transmission occurs. So following manipulation of the intracranial pressure, inner ear pressure decreases somewhat faster than it increases (Figure 3).

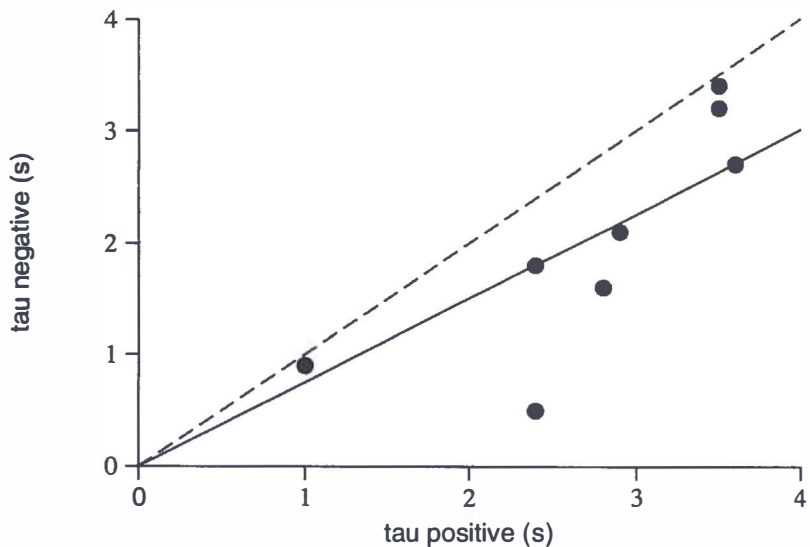


Figure 3 - Time constants of inner ear pressure change, for both increasing and decreasing inner ear pressure, after square wave-wise intracranial pressure manipulation. Time constants are smaller for pressure decrease (tau negative) than for pressure increase (tau positive).

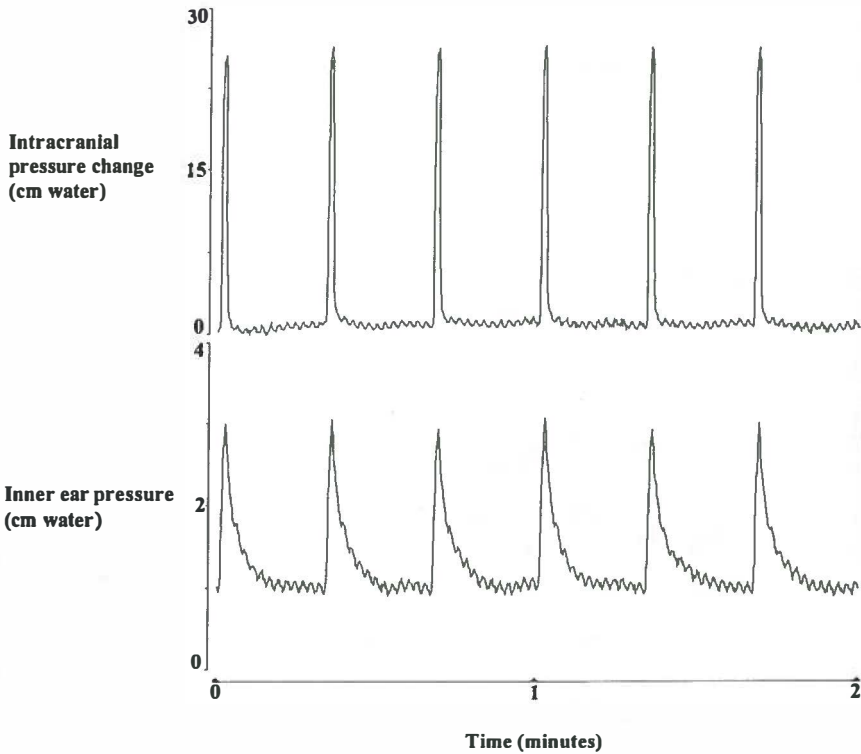


Figure 4 - Part of the results of pressure recordings in one animal: changes of inner ear pressure following pulse-wise manipulated intracranial pressure changes. Inner ear pressure immediately returns to its baseline value after cessation of the short pressure pulse.

Intracranial pressure manipulation with short rectangular pulses

The effect of rectangular pulse intracranial pressure manipulation was studied in 9 ears of 9 animals. As during low frequency square pressure steps, inner ear pressure followed manipulated intracranial pressure immediately, and dropped to its baseline value after cessation of the intracranial pressure pulse (Figure 4).

Except for 1 animal, pressure pulses were offered in series with increasing intensity. In total, we analysed the results of 23 series of pulse-wise pressure manipulations. The mean maximum inner ear pressure change was 1.94 cm water (range 0.35-8.47), following a mean maximum intracranial pressure change of 25.87 cm water (range 12.56-44.29). Fits were made to the declining parts of the inner ear pressure curves. In all experiments, best fits were obtained using the double exponential function, instead of the single exponential function. The mean time constant derived from the fits was 1.7 sec. (range 0.5-3.6). By

increase of inner ear pressure change, time constants decreased, indicating a faster pressure equalization in 6 guinea pigs (Figure 5).

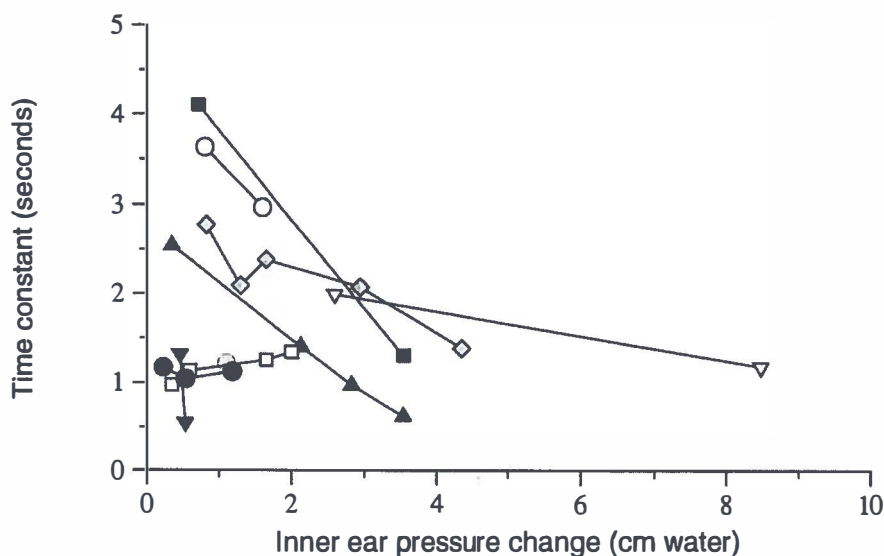


Figure 5 - Relation between measured time constants for inner ear pressure recovery after a short increase of intracranial pressure (as shown in Fig. 4) and the amplitude of inner ear pressure change. Different connected symbols are for different animals.

Sinusoidal intracranial pressure manipulation

The nonlinear behaviour of the inner ear pressure change is obvious in the following experiment: In one animal, intracranial pressure was manipulated also with a sinusoidal signal with a triangular envelope (Figure 6a). If the system had been linear, inner ear pressure change would have been proportional to the intracranial pressure change, which is clearly not the case (Figure 6b).

Perilymphatic potential

During inner ear pressure measurements, the perilymphatic potential remained stable.

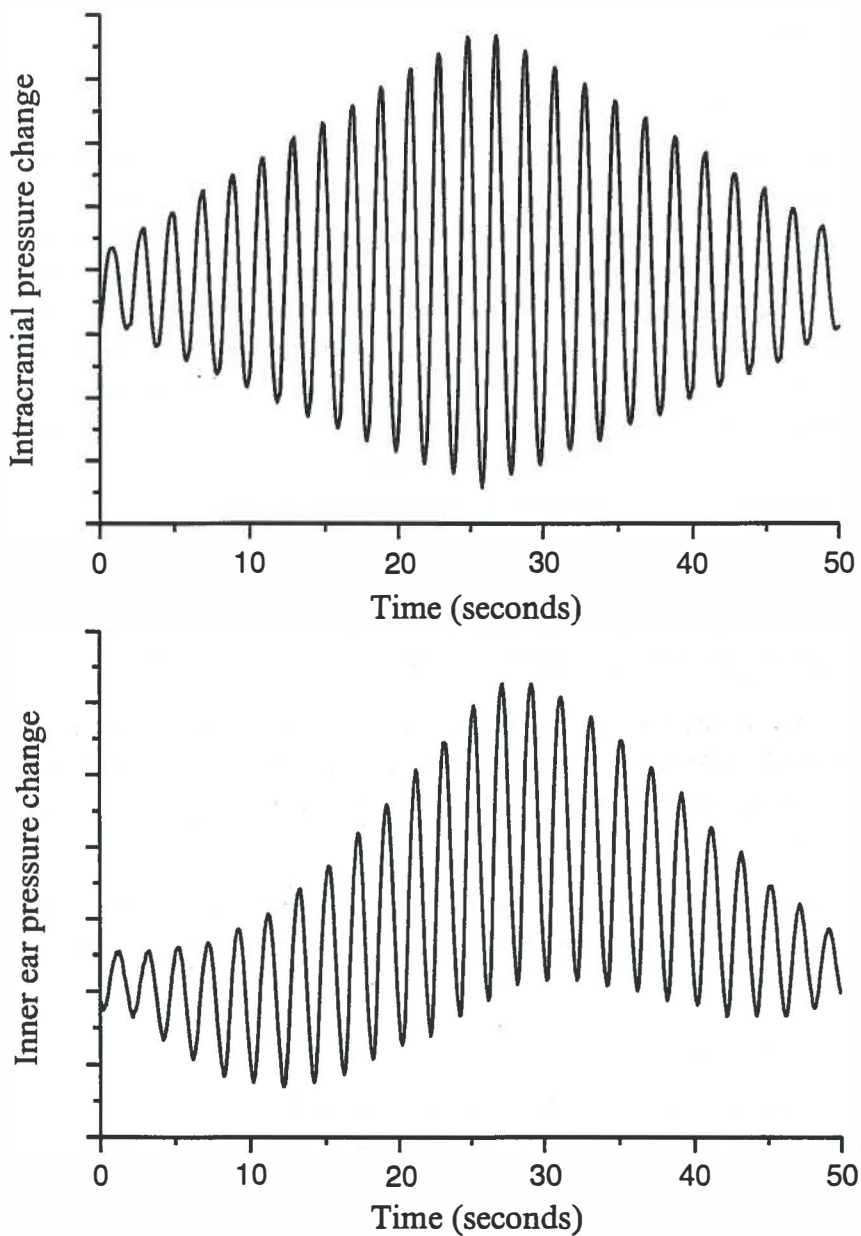


Figure 6a - Amplitude modulated intracranial pressure change (averaged over several periods of the envelope).

Figure 6b - Measured inner ear pressure response to the intracranial pressure change as is shown in Fig. 6a (averaged over several periods). The y-axis is enlarged, and adjusted to Fig. 6a.

Discussion

The cochlear aqueduct

In guinea pigs the cochlear aqueduct is the main connection between scala tympani and the intracranial compartment (4, 6), and it is funnel shaped. In man the aqueduct is comparatively long, narrows at its mid-point, and pursues a course separate from the inferior cochlear vein which lies more medial in Cotugno's canal (Mukherji et al. 1998). Its lumen is filled with a loose network of connective tissue, contiguous with the arachnoid membrane (Jackler and Hwang 1993; Toriya et al. 1991). The part of the fibrocyte network outside of the cochlear aqueduct stretches from the bony cochlea to its connection with the round window membrane. The loose network of connective tissue is denser at the connection to scala tympani. In light microscopy studies of the cochlear aqueduct of guinea pigs, this dense tissue has been called a valve (Toriya et al. 1991), or even a barrier membrane: the membrana limitans (Waltner 1948). On a histomorphological basis it is unlikely that fibrocytes would form a membrane, as they are of mesenchymal instead of ectodermal origin (Galic and Giebel 1987).

Until 1997, it was believed that the patency of the cochlear aqueduct in man decreased with increase of age (Wlodyka 1978). However, this finding could not be confirmed in a recent extensive study of the human cochlear aqueduct (Gopen et al. 1997).

According to our results, the cochlear aqueduct plays an important role in stabilizing inner ear pressure, being part of the system of inner ear pressure regulation.

Intracranial pressure manipulation

To obtain an inner ear pressure change of a few centimetres of water a 10 to 20-fold higher change of intracranial pressure was needed. Carlborg et al. (1980), however, when studying the relation between cerebrospinal fluid pressure and perilymphatic pressure in the cat did not find such a pressure difference. The essential difference between their and our approach is that we manipulated intracranial pressure extradurally at the top of the guinea pig skull, while in the cat Carlborg et al. manipulated and recorded cerebrospinal fluid pressure in one of the lateral ventricles, which was reached through the parietal bone. So it is quite

well possible that in our set-up a large pressure gradient exists across compliant intracranial structures between the top of the skull and the entrance of the cochlear aqueduct during intracranial pressure manipulation.

An increased intracranial pressure is transmitted to the inner ear by means of fluid inflow through the cochlear aqueduct (Carlborg et al. 1992; Marchbanks and Reid 1990). Pressure transients are influenced by the flow resistance of the cochlear aqueduct and the compliant nature of structures bordering the inner ear compartments. A permanently increased volume of inner ear fluid causes an increased inner ear pressure, which will lead to an outward movement of both the compliant round and oval window membranes into the middle ear. In transmitting middle ear pressure to the perilymphatic compartment the round window membrane is relative more important than the oval window membrane (Suzuki et al. 1998). Because the endolymphatic compartments in the normal guinea pig inner ear are bordered by highly compliant membranes, no pressure difference is measurable between endolymph and perilymph (Böhmer 1993; Takeuchi et al. 1990; Andrews et al. 1991; Yoshida and Uemura 1991). Apart from the cochlear aqueduct, there is an open connection between the inner ear and the intracranial compartment through the vestibular aqueduct and the perineural and perivascular spaces (Cotugno's canal). Inner ear pressure is not only influenced by the intracranial pressure, it is also affected by pressure in the middle ear and the external ear canal (ambient pressure). In experiments by Densert et al. (1986), the cochlear aqueduct was obliterated in cats. By means of middle ear pressure manipulation, inner ear pressure was changed. Although there was a difference in the amplitudes between these two pressure levels, the pressure change followed the same pattern in both the middle ear and the inner ear. The same experiment was performed in cats with an open cochlear aqueduct. In this case, inner ear pressure equalized to its baseline value within a few seconds after middle ear pressure change. Carlborg et al. (1982) also studied inner ear pressure change following manipulated external ear canal and middle ear pressure in cats, with the cochlear aqueduct open and blocked. He concluded that occlusion of the cochlear aqueduct reduced the compliance of the inner ear and severely reduced the pressure release capacity. In such a situation the inner ear is almost incapable of equilibrating ambient pressure changes. These experiments stress the importance of the cochlear aqueduct in equalizing inner ear pressure.

Carlborg et al. also showed a slight increase of inner ear pressure during increased intracranial pressure with an obliterated cochlear aqueduct. However this pressure change was not measurable during the first seconds after intracranial pressure was increased (Carlborg et al. 1982). The effect of pressure transfer routes other than the cochlear aqueduct is too slow to be of importance in our experiments, as we are studying inner ear pressure change during the first seconds of intracranial pressure change.

If the pressure equalization process of the inner ear had been linear, the simple exponential function should have given a perfect fit to the pressure curves of the inner ear (Figure 2a, 2b). Our results indicate that the pressure equalization process of the inner ear is nonlinear. This nonlinearity may be caused by a pressure dependent compliance of the round and oval window membranes (Suzuki et al. 1998). Or, when a constant compliance of the round and oval window membranes is assumed, our results indicate that the cochlear aqueduct behaves as a pressure dependent flow resistance.

In the latter case, this resistance is expected to depend on the amplitude of the intracranial pressure change (Figure 5). This dependence is possibly caused by the meshwork of cells in its lumen. This loose network of connective tissue might also explain the difference of flow resistance of the cochlear aqueduct for fluid motion in opposite directions. The cochlear aqueduct apparently provides a greater resistance to fluid motion into the inner ear compared to fluid motion out of the inner ear (Figure 3), which is in accordance with results obtained after injection of fluid into the inner ear (Wit et al. 1999). That the time constant for pressure equalisation through the cochlear aqueduct depends on pressure and flow direction was also found by Carlborg et al. (1982), who studied the influence of middle ear pressure changes on inner ear pressure in the cat.

As can be seen in Figure 3, the time constant for pressure increase correlates well with that for pressure decrease after sudden intracranial pressure change in the same guinea pig. However, time constants measured in different animals show large differences. This may be due to the anatomical differences of the cochlear aqueduct, its network of loose connective tissue and the compliance of the window membranes in different guinea pigs.

Inner ear pressure regulation is an important prerequisite for normal functioning of the inner ear. An increase of inner ear pressure has been proved to cause a

vestibular response (Sakikawa et al. 1999). In patients with Menière's disease the symptoms of tinnitus, paroxysmal vertigo and fluctuating hearing loss are believed to be related to an increase of inner ear pressure. These symptoms also occur in patients with intracranial hypertension (pseudotumor cerebri) and perilymphatic fistulae (Sismanis 1987). In man inner ear pressure is continuously influenced by ambient pressure changes and intracranial pressure changes (posture, breathing, swallowing), without causing any symptoms. Konradsson et al. (2000) studied pressure transfer between intracranial and labyrinthine fluids in patients with Menière's disease, with the tympanic membrane displacement (TMD) method. Their results indicate that for the patients tested, the routes of fluid communication were more effective in the diseased ear than in the healthy ear.

The cochlear aqueduct may be a canal with dynamic properties that can be related to clinical symptoms in man. Further research is required in order to obtain a better knowledge concerning the dynamics of inner ear pressure regulation and the role of the cochlear aqueduct therein. Maybe in the future a better treatment can be offered to patients suffering from the symptoms caused by intralabyrinthine pressure dysregulation.

Chapter 5

INNER EAR PRESSURE CHANGE FOLLOWING SQUARE WAVE INTRACRANIAL OR EAR CANAL PRESSURE MANIPULATION IN THE GUINEA PIG

*Thalen EO, Wit HP, Segenhout JM, Albers FWJ.
Inner ear pressure change following square wave intracranial or ear canal
pressure manipulation in the same guinea pig.
Eur Arch Otorhinolaryngol 2002; 259: 174-179*

Introduction

The homeostasis of inner ear fluids is essential for the functions of hearing and equilibrium. Fluctuating hearing loss, paroxysmal vertigo and tinnitus in patients with Menière's disease or intracranial hypertension are believed to be related to an increase of inner ear pressure (Sakikawa et al. 1994). A sudden pressure change within the inner ear can be corrected both by means of fluid in- or outflow through channels connecting the perilymphatic compartment with the intracranial fluid compartment, and the compliant nature of the membranous labyrinth. The main pressure transduction channel is the cochlear aqueduct (Carlborg et al. 1982; Carlborg 1992; Marchbanks and Reid 1990); the main compliant structure is the round window membrane (Ivarsson and Pedersen 1977).

The alternative situation can also occur, whereby changes of intracranial and external ear canal (EEC) pressure (atmospheric pressure) can cause a change of inner ear pressure. Studies have revealed daily variations of the intracranial pressure under normobaric conditions (Sakikawa et al. 1994). A temporary shift in intracranial pressure after neurosurgery can cause a temporary shift in hearing level (Walssted et al. 1994). The influence of ambient pressure changes on cochlear and vestibular function has been indicated in several reports of aerospace and underwater medicine, as well as in studies on the effects of pressure chamber treatment of Menière's disease (Densert et al. 1997; Konradsson et al. 1997).

Information regarding the pressure regulation mechanism of the inner ear can be obtained by studying the recovery process after acute pressure change. In this study inner ear pressure was measured and the recovery process studied during square wave manipulation of intracranial pressure, and subsequently EEC pressure in the same guinea pig. A sudden change of intracranial pressure causes a slower change of inner ear pressure, because of the high flow resistance of the cochlear aqueduct. Under this condition the intracranial – inner ear fluid system behaves as a low-pass filter. On the other hand, if EEC pressure is suddenly changed, this change is immediately transferred to the inner ear through the oval window. Inner ear pressure subsequently slowly returns to the equilibrium value, because fluid escapes through the cochlear aqueduct. Under this condition the system behaves as a high-pass

filter. In these two different cases the time constants for inner ear pressure change should be equal, if the flow resistance of the aqueduct and the compliance of the windows have constant values, independent of pressure, fluid flow direction etc.

To obtain more detailed information about the physiology of the inner ear pressure regulation in relation to normal and disturbed inner ear function, the present experiment was designed: comparison of time constants for pressure changes during intracranial or EEC pressure changes.

Materials and methods

Animal preparation

Eight female pigmented guinea pigs (Harlan Laboratories UK; 510-1070 g body weight), having normal pinna reflexes, were used in the experiments. General anaesthesia was induced by intramuscular administration of ketamine/xylazine (60/3.5 mg/kg). Muscle relaxation was obtained with succinylcholine (2.5 mg/kg). Tracheotomy was performed and ventilation was maintained by a respirator (Columbus Instruments, model 7950, breathing rate 40 per minute). Body temperature was kept stable at 38°C and the heart rate was monitored throughout the experiment. Each animal was placed in the supine position with the head kept stationary by means of a steel bolt fixed to the skull with dental cement. The tympanic bulla was opened by a retroauricular approach, and the round window membrane exposed. The care and use of the guinea pigs involved in this study were approved by the Animal Studies Committee of Groningen University.

Perilymphatic pressure measurement

Hydrostatic pressure in the perilymphatic compartment of the inner ear was measured through a micropipette using a servo-nulling micro pressure system (WPI model 900A). Micropipettes were drawn from borosilicate glass and the tips were bevelled to a diameter of approximately 5µm using a pipette grinder (Narishage EG-40). The micropipettes were filled with 2 M NaCl. By means of a micromanipulator the micropipette was inserted through the round window membrane into scala tympani, where the perilymphatic pressure was recorded. The DC-potential at the pipette tip was measured to verify its

position. A reference silver/silverchloride wire electrode was placed in the neck muscle of the guinea pig. Pressure calibration was done at the beginning of each experiment by lowering the pipette in saline over a known distance.

Intracranial pressure manipulation

In order to measure and manipulate intracranial pressure, two holes were drilled in the cranium of the guinea pig without causing damage to the dura. In these two holes short stainless steel tubes (1.5 mm diameter) were fixed with dental cement. A 1 mm diameter solid state pressure transducer (MTC P3FU catheter type HD 43002, B. Braun, Melsungen) was connected with one tube, and an infusion system filled with heparinised Ringer's solution was connected with the other tube. Low frequency (0.01 Hz) square wave IC pressure changes were obtained by means of an electronically controlled pneumatic system connected with the infusion system. (This system was in fact the pressure regulating part of a modified WPI 900A system.)

External ear canal pressure manipulation

In order to manipulate EEC pressure, a 3 mm (external) diameter silicone tube was inserted and airtight sealed in the EEC by means of Bison superglue®. Low frequency (0.02 Hz) square wave EEC pressure changes with varying amplitudes were obtained by means of the same electronically controlled pneumatic system, used for the intracranial pressure manipulation, connected with the silicone tube.

Data handling

During pressure manipulation pressure in the peridural space or the EEC and in the perilymphatic compartment of the inner ear, together with the DC-potential of scala tympani were stored digitally, with a rate of 10 Hz, after low-pass filtering (cut-off frequency 5 Hz). Recordings were processed off-line with an appropriate software package. Responses to repeated stimulus presentations were averaged and the signal component due to the breathing of the animal was removed from the response by smoothing its Fourier transform at the frequency of breathing.

Fits to the inner ear pressure equalization curves after a sudden intracranial

or EEC pressure change were made with different functions (Wit et al. 1999):

- I A single exponential function; this assumes that the inner ear fluid volume flow is proportional to the pressure difference between the inner ear and the intracranial compartment, and the inner ear pressure is a linear function of the inner ear fluid volume. These assumptions mean that aqueduct flow resistance and window compliance are constants.
- II A “double exponential” function; this assumes that the change of pressure with time during pressure recovery is a second order function of pressure. As a consequence, flow resistance and/or compliance are no longer constants in this case.
- III A hyperbolic function; this is a special case of formula II, for a purely quadratic relation between pressure and the change of pressure with time.

Time constants were derived from the fits. Time constants following intracranial or EEC manipulation were compared.

Results

Successful experiments were performed in ten ears of eight guinea pigs. In five ears of five guinea pigs both EEC and intracranial pressure were manipulated and measured. In three ears of three guinea pigs only EEC pressure was manipulated successfully and in two ears of two guinea pigs only intracranial pressure was manipulated successfully. The mean steady state pressure in the perilymphatic compartment of the inner ear was 4.6 cm water (range 2.7-6.0).

Intracranial pressure manipulation

An illustrative result of an inner ear pressure measurement during intracranial pressure manipulation is given in Figure 1. Inner ear pressure immediately follows a change in intracranial pressure, and stabilizes within a few seconds. The inner ear pressure curve is a low-pass filtered version of the intracranial-pressure profile. A mean pressure step of intracranial pressure of 29.8 cm water (range 16.5-49.5) induced a mean change of inner ear pressure of 1.4

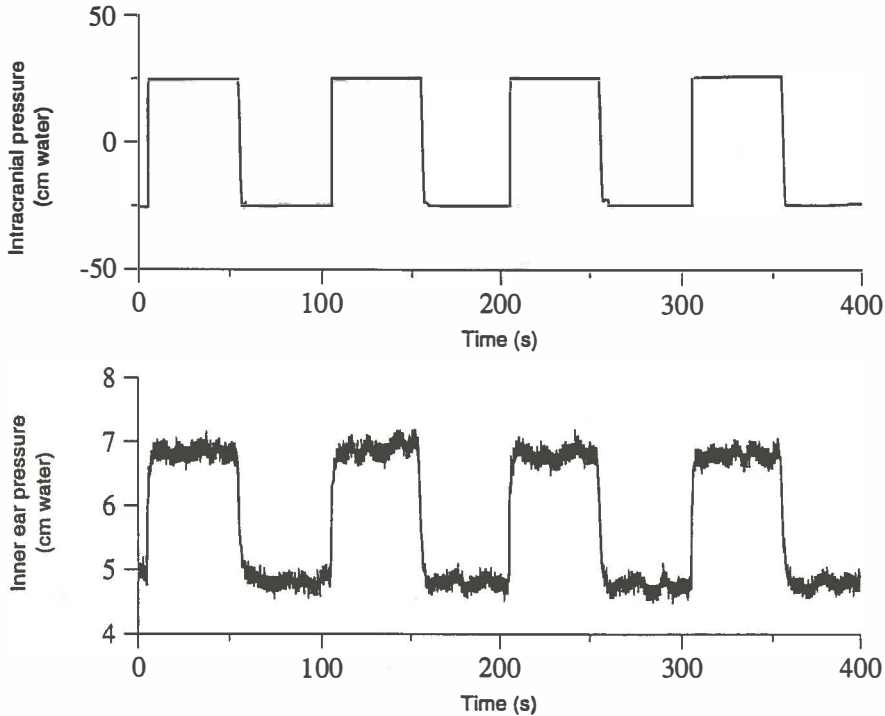


Figure 1 - Part of a measurement in which intracranial pressure (upper panel) was manipulated with low frequency square wave pressure steps. Inner ear pressure in the perilymphatic compartment (lower panel) follows without a measurable delay; the system behaves as a low-pass filter.

cm water (range 0.6-2.6). Fits were made to the up and down going slopes of the inner ear pressure curves and time constants were derived from the fits. Time constants for inner ear pressure decrease (fluid flow out of the cochlea; mean $\tau = 2.3$ s range 0.94-3.84) were smaller than time constants for inner ear pressure increase (fluid flow into the cochlea; mean $\tau = 3.2$ s, range 0.99-5.46), in the same guinea pig (Figure 2). Best fits were obtained for all measurements using the hyperbolic function (formula III).

EEC pressure manipulation

An illustrative result of an inner ear pressure measurement during EEC pressure manipulation is given in Figure 3. After an increase or decrease of EEC, inner ear pressure follows manipulated EEC pressure immediately, without a measurable time lag in the onset of pressure change. The initial pressure change of the inner ear is followed by a pressure recovery to its initial value, as in a high-pass filtering system.

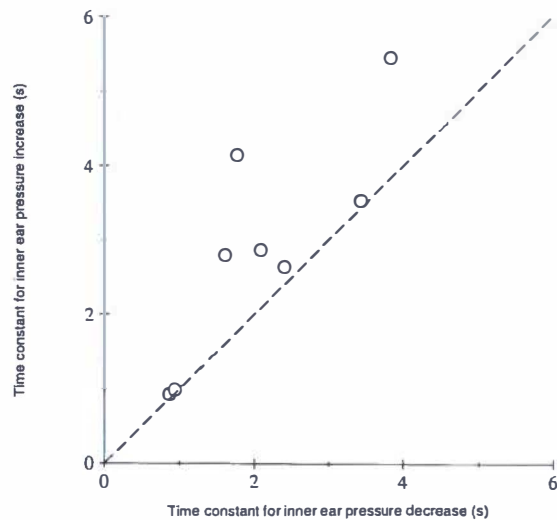


Figure 2 - Time constants for inner ear pressure equalization during pressure increase or decrease following manipulated intracranial pressure: pressure decrease occurs with a smaller time constant.

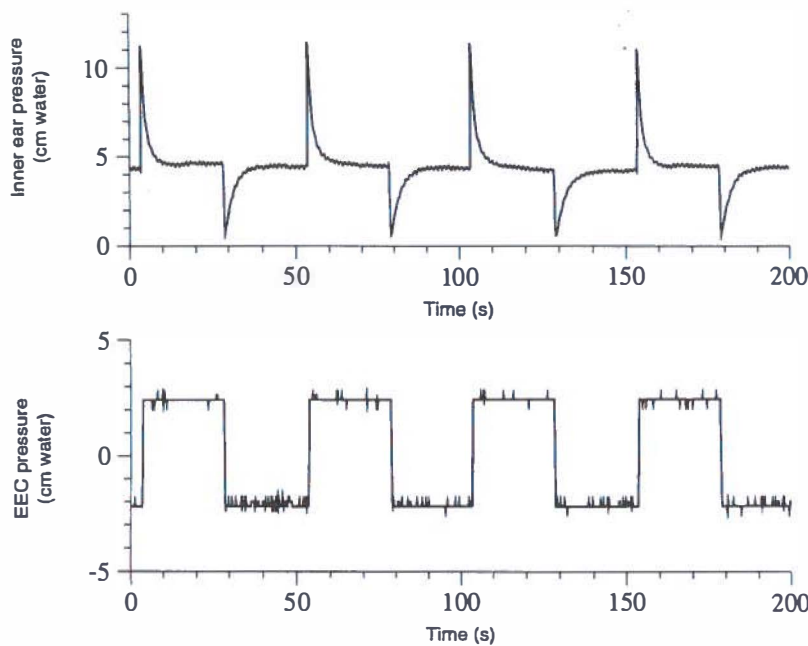


Figure 3 - Part of a measurement in which external ear canal pressure (lower panel) was manipulated with low frequency square wave pressure steps. Inner ear pressure in the perilymphatic compartment (upper panel) follows without a measurable delay; the system behaves as a high-pass filter.

A mean upward pressure step in the EEC of 4.3 cm water (range 0.8-11.5) was followed by a mean maximum positive pressure change of the inner ear of 3.6 cm water (range 0.8-12.1) while a smaller mean maximum negative pressure change of the inner ear of 2.4 cm water (range 0.7-5) was the result of the returning 4.3 cm water downward pressure step (range 0.1-7.7). This asymmetry in the inner ear pressure response was obvious in all measurements. Best fits to the inner ear pressure equalization curves were obtained with formula II, the “double exponential” function. The mean time constant was 1.72 seconds (range 0.51-2.73) after EEC pressure increase, and 2.85 seconds (range 0.93-4.14) after EEC pressure decrease. In the same guinea pig time constants for inner ear pressure decrease (fluid flow out of the cochlea) were smaller than time constants for inner ear pressure increase (fluid flow into the cochlea) (Figure 4). In seven of the eight ears

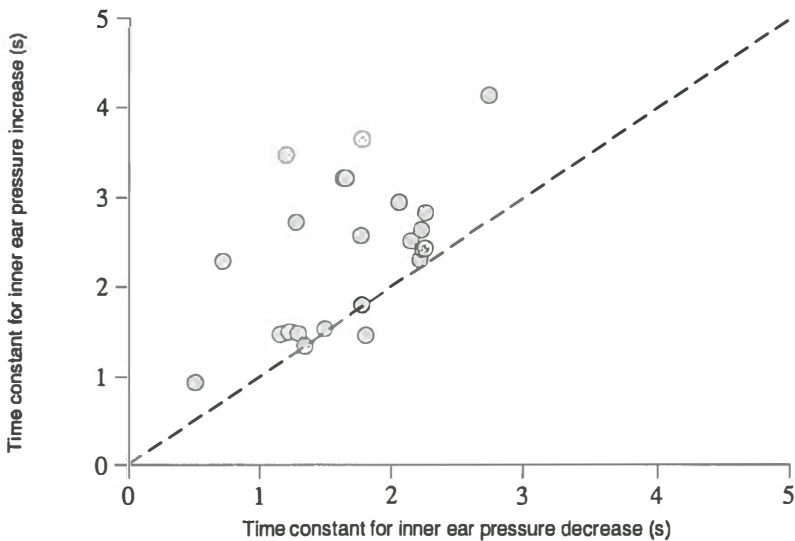


Figure 4 - Time constants for inner ear pressure equalization during inner ear pressure increase or decrease following manipulated external ear canal pressure: inner ear pressure decrease (fluid flow out of the cochlea) occurs with a smaller time constant.

EEC pressure was manipulated with differing intensity steps. In some of these ears the ratio of time constants for pressure decrease or increase depended on the initial EEC pressure change; in others it did not (Figure 5).

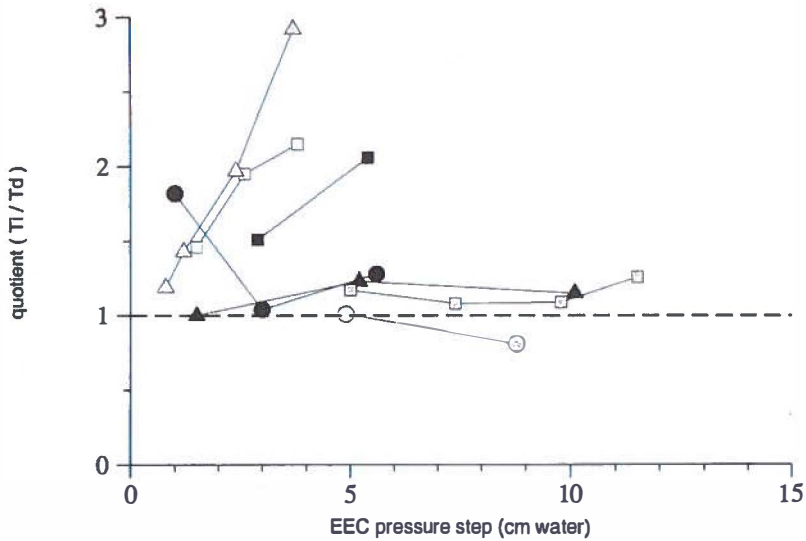


Figure 5 - Time constants for pressure increase (T_i) divided by time constants for pressure decrease (T_d), for different values of the amplitude of ear canal pressure change (connected symbols represent measurements in the same ear).

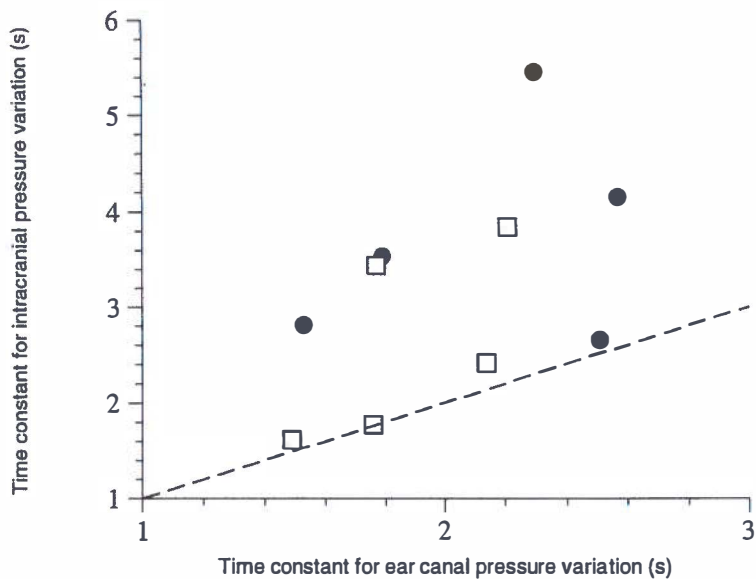


Figure 6 - Comparison of time constants for inner ear pressure equalisation following intracranial versus external ear canal pressure manipulation in the same guinea pig (filled circles represent time constants for fluid flow into the cochlea, squares represent time constants for fluid flow out of the cochlea).

Pressure equalization following intracranial versus EEC manipulation

In five ears of five guinea pigs both intracranial and EEC pressure manipulations were performed successfully. A comparison was made between EEC and intracranial steplike pressure variations which resulted in inner ear pressure variations with comparable amplitudes. Time constants for these pairs of inner ear responses are given in Figure 6, both for fluid flow out of the cochlea after inner ear pressure increase and for fluid flow into the cochlea after inner ear pressure decrease.

Discussion

For small increases or decreases of EEC pressure inner ear pressure responses were symmetric and could satisfactorily be fitted with a single exponential function. However, for larger EEC pressure changes, inner ear pressure responses became asymmetric, and fits with a single exponential function gave a systematic deviation from the measured pressure equalisation curves (Figure 7a).

Without exception, perfect fits to the curves were obtained with the double exponential function (Figure 7b). Best fits to the inner ear pressure equalisation curves, following intracranial pressure manipulation, were obtained with the hyperbolic function. Both for ear canal, and intracranial pressure manipulation, inner ear pressure changes could best be described with a nonlinear model for pressure equalisation. The consequence of this is that the flow resistance of the cochlear aqueduct and/or the compliance of the membranes bordering the inner ear do not have a constant value. This was also concluded by Densert et al. (1978) and by Carlborg et al. (1982), based on pressure equalisation experiments in cats.

In all experiments described in the present study, fluid flow out of the cochlea is somewhat faster than fluid flow back into the cochlea (Figures 2 and 4). This finding is in accordance with a previous study, in which intracranial pressure was manipulated with different intensities (Thalen et al. 2001). The most straightforward explanation for the difference in time constants for different flow directions is that the flow resistance of the cochlear aqueduct depends on flow direction. During EEC pressure manipulation not only the time constants following pressure increase or decrease are different (Figures

4 and 5), but also the initial amplitude of the inner ear pressure response following a positive EEC pressure change was larger than for a negative pressure change (Figure 3).

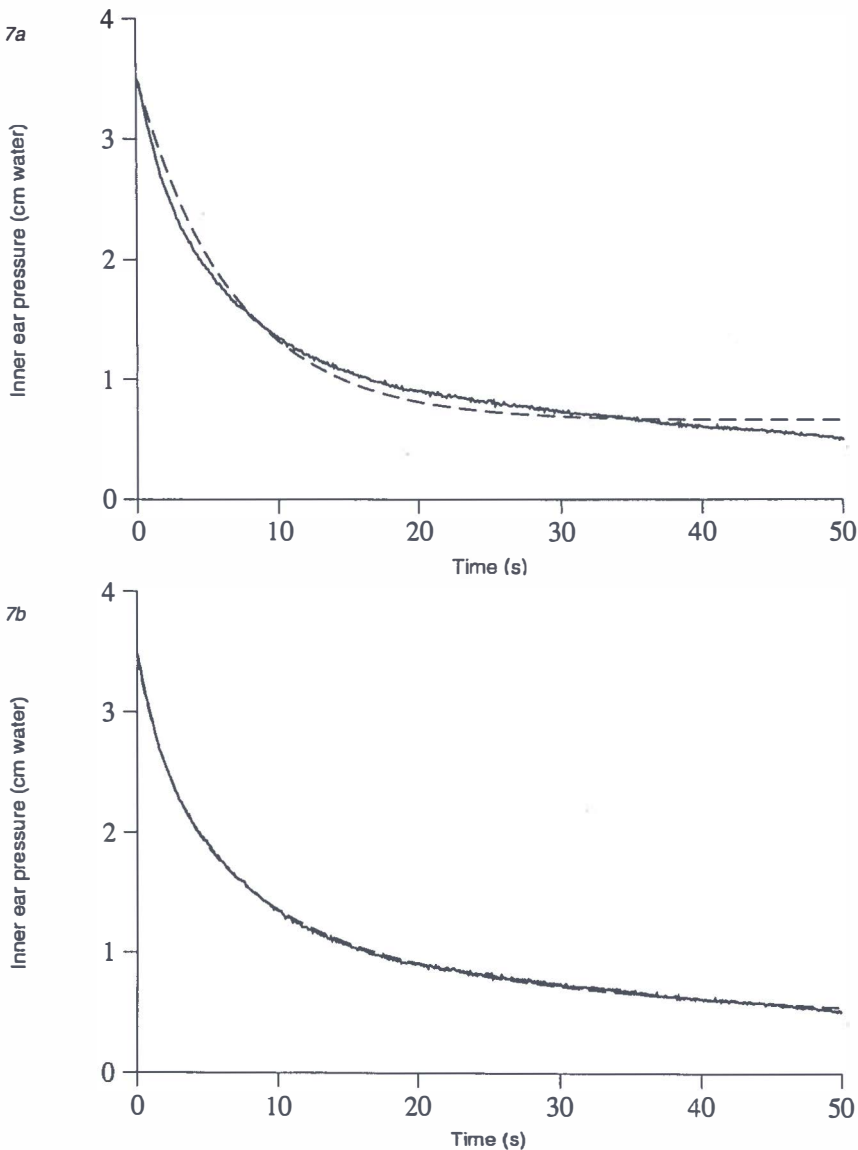


Figure 7a - Pressure equalisation curve after a pressure step in the external ear canal (continuous line) fitted with an exponential function (dashed line).

Figure 7b - The same curve as in Figure 7a now fitted with a "double exponential function".

A similar asymmetry in response amplitudes after external ear canal pressure change in guinea pigs was found by Nishihara et al. (1992). To explain the difference, these authors assume that the tympanic membrane moves more easily inwards than outwards, when a static pressure is applied to the external ear canal. This assumption, however, cannot explain our results, as the resting position of the tympanic membrane would then have changed after each square wave (up and down) pressure step, leading to a tympanic membrane that would have moved far inward after a series of such steps, as applied in Figure 3.

An explanation for the observed asymmetry in response amplitudes can not easily be given. The relation between ear canal pressure and the force working on the stapes is not straightforward: the tympanic membrane is not a rigid plate and its connection to the stapes is also not rigid. This is in fact a drawback of the present study. Results of direct middle ear pressure manipulation, with a perforated tympanic membrane, are more easily interpreted than results of ear canal pressure manipulation with an intact tympanic membrane (Feijen et al. 2000).

During intracranial pressure manipulation, the pressure changes measured with the peridural positioned pressure transducer were much larger than the perilymphatic pressure changes caused by it. Intracranial pressure manipulation was performed by a change of extradural fluid volume at the top of the guinea pig skull. The cochlear aqueduct, however, ends in the subarachnoid space near the meatus acousticus internus, lateral in the skull base. It is possible that in our system, a large pressure gradient exists across compliant intracranial structures between the top of the skull and the exit of the cochlear aqueduct.

Comparing inner ear pressure profiles for square wave EEC, or intracranial pressure changes shows, as expected, that these profiles are complementary: inner ear pressure follows ear canal pressure immediately and returns slowly to its equilibrium value, as in a high-pass filtering system, while for intracranial pressure change inner ear pressure follows slowly, as in a low-pass filter. Time constants for both ways of pressure manipulation are comparable, but not equal, showing that the experimental results can not be described with a simple linear model. This is underlined by the fact that no obvious relation

exists between time constants of inner ear pressure change following intracranial or EEC pressure change (Figure 6), when measured in the same ear. The only clear similarity between the two methods of pressure manipulation is the fact that time constants for fluid flow out of the cochlea are smaller (faster fluid flow) than time constants for the opposite flow direction.

The nonlinearity of the pressure equalisation process of the inner ear, and the postulated direction dependency of the flow resistance of the cochlear aqueduct might be the result of special properties of the cochlear aqueduct and the periotic duct. The cochlear aqueduct is a narrow funnel shaped bony canal, which connects the scala tympani with the intracranial compartment at the posterior cranial fossa, inferior to the internal auditory canal and just superior to the jugular foramen. Inside the cochlear aqueduct is the periotic duct; a loose mesh of connective tissue that is contiguous medially with the arachnoid membrane and laterally with the periotic layer of scala tympani and the round window membrane in the basal turn of the cochlea (Jackler and Hwang 1993; Marchbanks and Reid 1990). In previous studies this periotic duct has been called a barrier membrane (Waltner 1948) or a valve (Toriya et al. 1991). Wlodyka (1978) showed that the cochlear aqueduct in humans loses permeability with increasing age, creating some doubt on the importance of the cochlear aqueduct for inner ear pressure regulation. However, a more recent study by Gopen et al. (1997) could not confirm Wlodyka's finding. Other channels connecting the perilymphatic compartment with the intracranial compartment are the vestibular aqueduct, perineural and perivascular spaces (Cotugno's canal) and the tympanomeningeal or Hyrtl's fissure (Jackler and Hwang 1993). Carlborg et al. (1982) and later Suzuki et al. (1994) have shown that these other transduction routes are less important for inner ear pressure equalisation.

When inner ear pressure is increased through increase of ear canal pressure (and the bulla is open), the round window will bulge outwards (Nishihara et al. 1992). The round window will also bulge outwards when extra fluid enters the inner ear through the cochlear aqueduct in case of increased intracranial pressure. As the periotic duct is connected both with the round window membrane and the bony wall of the cochlear aqueduct, it will be stretched. This stretching might cause a change of flow resistance of the cochlear aqueduct, and be one of the factors in the complicated behaviour of inner ear

pressure release. Seo et al. (1997) investigated the relation in humans' between the patency of the cochlear aqueduct (histologically studied post mortem) and clinical symptoms such as hearing disorders or disequilibrium (studied from the clinical charts). They stated that the patency of the cochlear aqueduct was significantly smaller in patients with clinical symptoms than in patients without symptoms. This suggests that the physiological function of the cochlear aqueduct in inner ear pressure regulation is of importance for a normal functioning of the inner ear.

Chapter 6

**DIRECT MEASUREMENT
OF
THE FLOW RESISTANCE OF THE COCHLEAR AQUEDUCT
IN THE GUINEA PIG**

*Thalen EO, Wit HP, Segenhout JM, Albers FWJ.
Direct measurement of the flow resistance of the cochlear aqueduct in the
guinea pig
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7

Introduction

The volume regulation mechanism of the inner ear fluids is important for the normal functioning of the inner ear. Disturbance of inner ear volume and pressure is thought to cause clinical symptoms such as vertigo, tinnitus and fluctuating loss of hearing. These symptoms can be observed in patients with perilymphatic fistulae (Böhmer 1991) or in patients with Menière's disease, where an endolymphatic hydrops is thought to be the histopathological substrate of the disease.

The cochlear aqueduct connects the scala tympani with the subarachnoid space. Horner (1993) stated that patients with small cochlear aqueducts had more audiovestibular complaints than patients with larger open cochlear aqueducts, which suggests a role for the cochlear aqueduct in inner ear pressure equilibration. To investigate the role of the cochlear aqueduct, Carlborg et al. studied inner ear pressure recovery after manipulation of middle ear and intracranial pressure in cats (Carlborg et al. 1982, 1983, 1992). In more recent experiments, we studied inner ear pressure changes during intracranial pressure manipulation (square wave- and pulse wise) and during both intracranial or external ear canal pressure manipulation in guinea pigs (Thalen et al. 2001, 2002). After sudden pressure change in the inner ear, pressure recovers to the baseline value within a few seconds. The time constant of this pressure recovery is determined by the flow resistance of the cochlear aqueduct and by the compliance of the window membranes, of which the round window membrane is the most compliant (Ivarsson and Pedersen 1977; Ishii et al. 1995).

In the experiments described above values for the product of flow resistance and window compliance were obtained from the measured time constants for pressure recovery after induced pressure changes. To obtain a value for the flow resistance of the aqueduct from this product window compliance has to be estimated. The present study was designed to determine the flow resistance of the cochlear aqueduct directly, in order to verify the estimates from earlier experiments.

Materials and methods

Six albino guinea pigs (Harlan Laboratories UK; 535-750g body weight) with adequate Preyer's reflexes were used in the experiments. General anaesthesia was induced by intramuscular administration of ketamine/xylazine (60/3.5 mg/kg). Muscle relaxation was obtained with succinylcholine (2.5 mg/kg). The animals were artificially ventilated (Columbus Instruments, model 7950) through a tracheostoma, and body temperature was kept stable at 38°C with a heating pad. Heart rate was monitored through subcutaneous needle electrodes. Each animal was placed in the supine position with the head kept stationary by means of a steel bolt fixed to the skull with dental cement. Following a retro-auricular approach, the tympanic bulla was opened and the round window membrane exposed. Hydrostatic pressure in the perilymphatic compartment of the inner ear was measured through a micropipette, using a servo-nulling micro pressure system (WPI model 900A). Micropipettes were drawn from borosilicate glass and their tips were bevelled to a diameter of approximately 5 μm using a pipette grinder (Narishage EG- 40). The micropipettes were filled with 2 M NaCl. The micropipette was inserted through the round window membrane into the scala tympani using a micromanipulator, where the perilymphatic pressure was recorded. The DC potential at the pipette tip was measured to verify its position. A reference silver/silver chloride wire electrode was placed in the neck muscle of the guinea pig. Pressure calibration was carried out at the beginning of each experiment by lowering the pipette into saline over a known distance. Continuous infusion of artificial perilymph into the scala tympani was performed through a second bevelled micropipette, with a tip diameter of approximately 30 μm . This tip diameter was a compromise between sufficiently low flow resistance for fluid injection (or withdrawal) and tip smallness. This micropipette, held in a second micromanipulator and connected to a reservoir filled with Ringer's solution, was also inserted into the scala tympani by perforating the round window membrane. Ringer's solution was infused at a constant rate by lifting the reservoir until a steady-state perilymphatic pressure was achieved. After a few minutes the reservoir was lowered to its original position and the perilymphatic pressure returned to its baseline value (see figure 1). This process was repeated for different rates of infusion. The fluid injection rate was calculated as the total injected volume divided by the total injection time. The injected volume was measured as the

displacement of the fluid meniscus in the tube connecting the reservoir to the micropipette, of which the inner diameter was precisely known (0.732 mm). By initially lowering the artificial perilymph reservoir, fluid was drawn from the inner ear. This process was also repeated for different flow rates.

During pressure manipulation, hydrostatic pressure in the perilymphatic compartment of the inner ear was monitored and the data stored digitally at a rate of 10 Hz, after low-pass filtering (cut-off frequency 5 Hz). Recordings were processed off-line with an appropriate software package. The care and use of the guinea pigs reported on in this study was approved by the Animal Studies Committee of the University of Groningen (protocol number DEC-1286).

Results

In six ears of the total group of six guinea pigs, both injection and withdrawal of artificial perilymph were performed successfully. The mean steady-state pressure in the perilymphatic compartment of the inner ear was 2.8 cm water (280 Pa; range 60-390). An illustrative result of an inner ear pressure measurement during fluid injection is given in Figure 1.

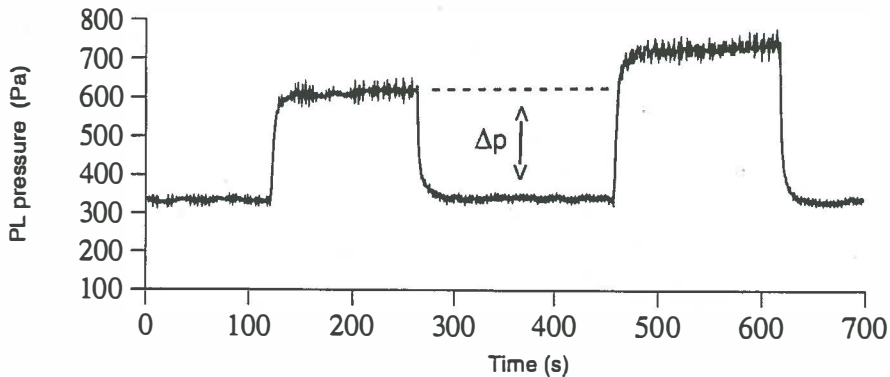


Figure 1 - Recording of perilymphatic pressure during infusion with two different rates. Between infusions, pressure returns to a baseline value of 330 Pa (= 3.3 cm water).

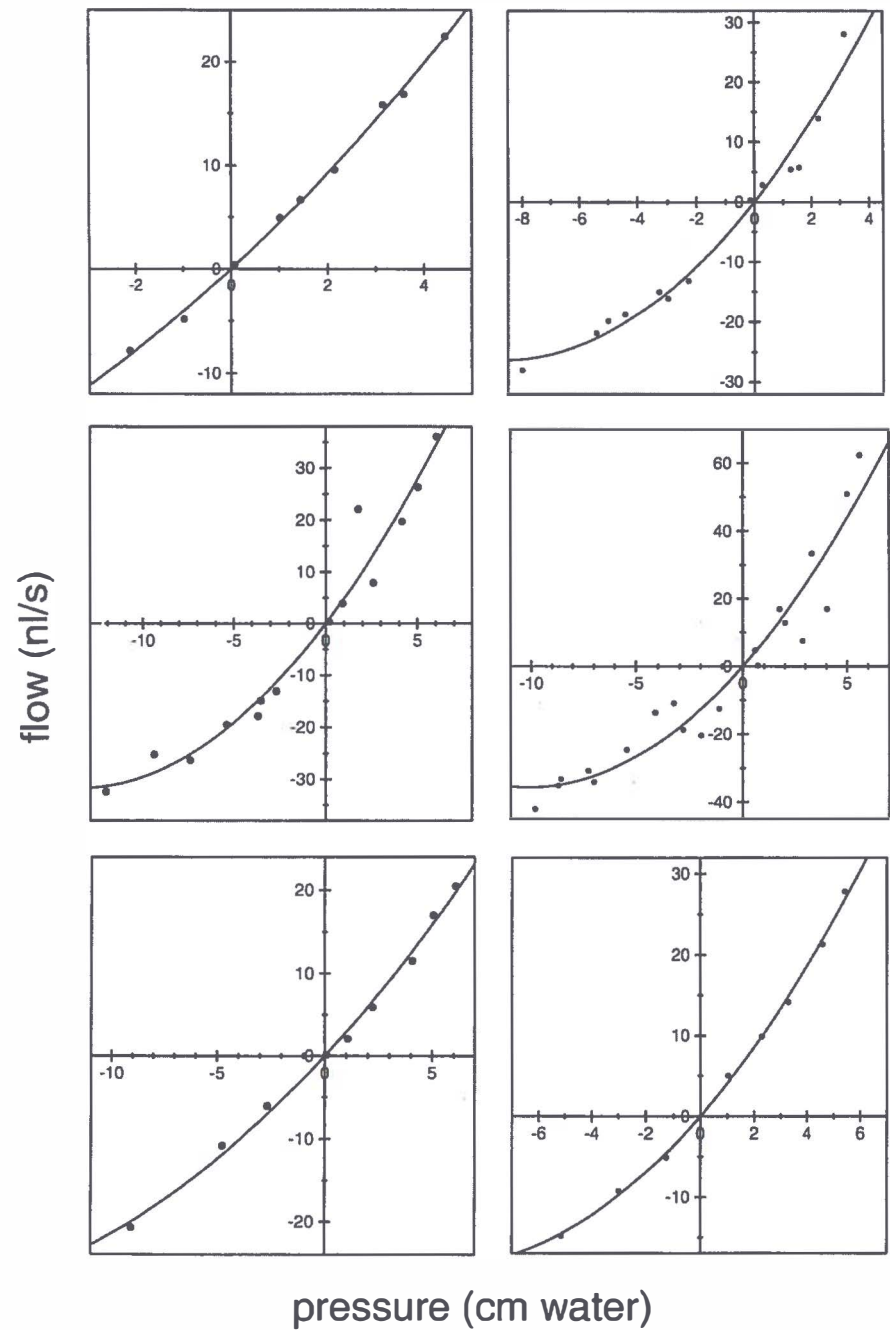


Figure 2 - Flow rates separately plotted against steady-state pressure increase or decrease Δp (in cm water) during infusion (see Figure 1) for all 6 guinea pigs. Solid lines: least-squares fits with second order curves to data points (black dots).

During fluid injection, perilymphatic pressure increases quickly until it reaches a new constant value. In this situation, injection rate is equal to the rate of fluid flow through the cochlear aqueduct. The various constant values for pressure increase Δp achieved in this way ranged from 10 to 1070 Pa (not all in the same guinea pig). Similarly, constant values for perilymphatic pressure decrease Δp ranged from 140 to 1310 Pa. Flow ranged from 0.4 to 62.4 nl/s during fluid infusion into the scala tympani and from 4.8 to 42.1 nl/s during fluid withdrawal.

In Figure 2, flow rates have been separately plotted against steady-state pressure increase or decrease during infusion for all six guinea pigs. As the relation between flow and pressure can be seen to be nonlinear, the data were least-squares fitted with second order curves. By dividing pressure Δp by the quadratic expression for flow as a function of Δp , an expression was obtained for the flow resistance R as a function of Δp . These relations are shown in Figure 3 for all six guinea pigs, together with a curve for the average resistance.

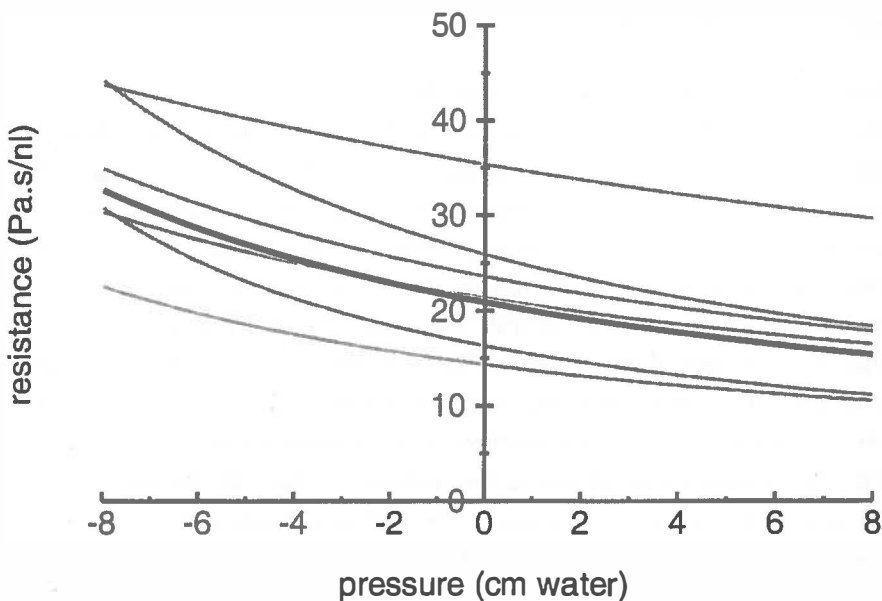


Figure 3 - Grey lines: flow resistance R as a function of Δp for all 6 guinea pigs. Black line: average of 6 individual results.

Discussion

In this experiment, aqueduct flow resistance R was directly measured. R can also be estimated from the results of earlier experiments, where the time constant τ for inner ear pressure release after a sudden change of inner ear pressure was measured. These experiments gave τ -values between 0.9 and 5.4 seconds (Thalen et al. 2001, 2002). For constant aqueduct flow resistance R and constant window compliance C the resistance R can be calculated with $\tau = RC$, if C is known. As far as we know, C , which is the sum of the round and oval window compliances, has never been directly measured in a guinea pig, but it can be estimated to have an average value of 0.17 nl/Pa (10). Combining this value for C with the above given range for τ yields a value for R between 5 and 32 Pa.s/nl. Given the uncertainty in the value for C , this range of estimated values for R is in good agreement with the directly measured values as shown in Figure 3. The accuracy of the measured relation between flow and Δp , as shown by the spread of the data points in Figure 2, does not allow for more than a qualitative analysis of this relation and its consequence, as shown in Figure 3.

Figure 2 shows that the flow through the aqueduct is not a linear function of the pressure difference Δp across it. This means that the flow resistance R of the cochlear aqueduct is not a constant, as can also be seen in Figure 3. By analysing inner ear pressure release after a sudden change of middle ear pressure (Feijen et al. 2002; Wit et al. 2003), evidence has been found for the dependence of aqueduct flow resistance on the round window position – the more the window bulges inwards the higher the resistance is. This could explain the shape of the curves in Figure 3: the position of the round window depends on Δp during fluid injection or withdrawal. For high pressure change during injection ($\Delta p > 0$), the window bulges outwards and R is relatively low. For high pressure change during fluid withdrawal ($\Delta p < 0$), the window bulges inwards and R increases. However, the middle ear pressure variation study mentioned above also showed that R does not depend on round window position alone; Δp itself also directly influences the flow resistance of the aqueduct. To complicate things even further, the dependence of R on the direction of fluid flow through the aqueduct (Wit et al. 1999; Thalen et al. 2001, 2002) cannot be excluded.

Influences of round window position, pressure difference and the direction of fluid flow are likely if structures inside the aqueduct and at its entrance in the scala tympani are taken into account. The cochlear aqueduct itself is a bony, funnel-shaped canal connecting the scala tympani of the basal turn of the cochlea with the subarachnoid space of the posterior cranial fossa (Ghiz et al. 2001). Its internal orifice is situated very close to the round window. Within its lumen is the periotic duct, a meshwork of fibroblasts and loose connective tissue contiguous with the cell network of the subarachnoid space, and connected to the round window membrane (Nishimura et al. 1981; Galic and Giebel 1987; Toriya et al. 1991). An outbulging position of the round window membrane causes traction to the periotic duct, which might increase the permeability of the CA. If the round window moves inwards, the flow resistance of the structures that connect the orifice of the aqueduct to the round window may increase. Furthermore, flow magnitude and direction may influence the shape of the structures inside the aqueduct. Detailed morphological examination of the cochlear aqueduct with relation to the round window membrane could contribute to a further understanding of this interesting and relevant mechanism.

Chapter 7

**THE COCHLEAR AQUEDUCT OF THE GUINEA PIG:
A MORPHOLOGICAL STUDY WITH EMPHASIS
ON INNER EAR PRESSURE REGULATION**

*Thalen EO, Wit HP, Segenhout JM, Albers FWJ.
The cochlear aqueduct of the guinea pig: a morphological study with
emphasis on inner ear pressure regulation.
Submitted*

Introduction

The cochlear aqueduct (CA) is a bony canal which connects the perilymphatic space of the scala tympani with the subarachnoid space at the posterior cranial fossa (Toriya et al. 1991). It is funnel shaped, narrows at its mid-point, and pursues a course separate from the inferior cochlear vein which lies more medial in Cotugno's canal (Mukherji et al. 1998). In the guinea pig the CA has a length of approximately 2 mm and its cross-sectional area increases from 0.016 mm² at the side of the scala tympani to more than 0.20 mm² at the posterior cranial fossa (Ghiz et al. 2001). Inside the lumen of the CA is the periotic duct, a meshwork of connective tissue. The periotic duct is not completely permeable as macrophages and erythrocytes have been found in it, not able to pass through. The fibrocyte meshwork inside the CA is contiguous with the arachnoid membrane and is connected outside the cochlear aqueduct with part of the round window membrane. The cellular components of the periotic duct are denser in the opening to the perilymphatic space than in the duct portion (Duckert 1974; Nishimura et al. 1981; Toriya et al. 1991; Jackler 1993).

The main route for pressure equalization of the inner ear is the cochlear aqueduct (Carlborg et al. 1992). Pressure equalization by means of fluid flow takes place within seconds after a sudden change of inner ear pressure, for instance by a change of ear canal (Krukowski et al. 1980) or middle ear pressure (Densert et al. 1981) or by injection of fluid into the inner ear (Wit et al. 1999). The flow resistance of the cochlear aqueduct not only depends on the fluid flow direction through the aqueduct (Thalen et al. 2002), but is also influenced by the pressure difference across the aqueduct (Thalen et al. 2001; Feijen et al. 2002; Wit et al. 2003).

The change of the flow resistance during inner ear pressure variation and variation in flow across the cochlear aqueduct can be explained by a permeability change of the cochlear aqueduct, caused by specific morphological characteristics of the cochlear aqueduct and its entrance in the scala tympani. This light microscopic study was designed to investigate the morphology of the cochlear aqueduct in relation to its dynamic properties.

Material and methods

Five healthy adult guinea pigs (Harlan, the Netherlands) with adequate Preyer's reflexes were used in this study. Animal care and use were in accordance with the principles of the declaration of Helsinki and approved by the Groningen animal experiment committee (protocol number DEC 2830). Animals were terminated by means of intracardial administration of an overdose pentobarbital. Both temporal bones and the attached dura mater were dissected out. The temporal bones were fixated by immersion in a solution of 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer (pH 7.4; 4°C; 400Mosm) and 2mM calcium chloride. After fixation, the samples were decalcified for five days in 10% EDTA (pH 7.4), carefully rinsed in distilled water, dehydrated in a graded ethanol series and embedded in plastic. Serial cross-sections (1-2.5µm thick) were cut perpendicular to the long axis of the CA in two guinea pigs, and parallel to the long axis of the CA in three guinea pigs. All cross-sections were prepared with a microtome (Microm HM 350).

The sections of the CA were stained with toluidine blue in combination with basic fuchsin for light microscopic examination (Leica DM/RX-A).

Results

Serial cross-sections were cut perpendicular to the long axis of the CA in two guinea pigs and parallel to the long axis of the CA in three guinea pigs. The main duct has a flattened funnel shape. Its narrowest portion is just before its opening to the perilymphatic space. At the opening to the perilymphatic space, the diameter of the duct increases slightly from the narrowest portion, then decreases, and subsequently the duct opens to the perilymphatic space (elongated pear-like shape). The direction of the opening of the CA has an angle of approximately 30 degrees with the direction of the main duct (Figure 1). An estimation of the radius of the opening of the cochlear aqueduct into the scala tympani is dependent of the locus that is chosen to be the end of the aqueduct. The bony part of the opening is elliptical, whereas the periotic duct thins out further into the perilymphatic compartment. The smallest diameter of the (bony) cochlear aqueduct at its opening to the scala tympani, is approximately 120 µm.

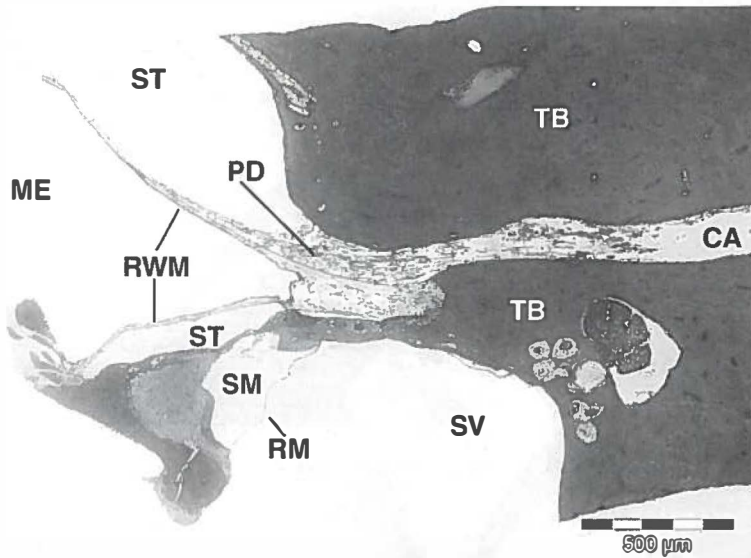


Figure 1 - Longitudinal cross-section of the cochlear aqueduct (CA) and its connection with the scala tympani (ST). Inside its lumen the periotic duct (PD), which is connected with the round window membrane (RWM). Other structures are the middle ear (ME), scala vestibuli (SV), Reissner's membrane (RM), temporal bone (TB)

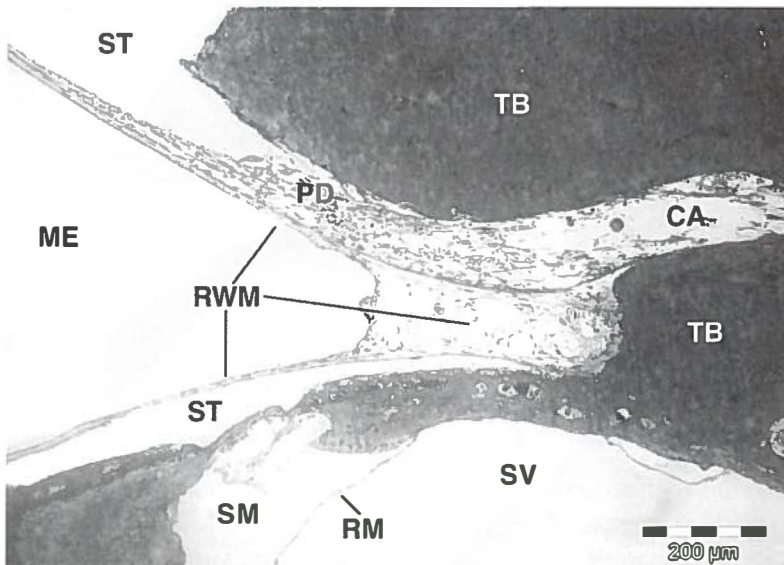


Figure 2 - The opening of the cochlear aqueduct (CA) into the scala tympani (ST): the cell meshwork of the periotic duct (PD) is more dense at the opening to the scala tympani (ST). Other structures are the round window membrane (RWM), round window membrane extension (RWME), middle ear (ME), scala media (SM), scala vestibuli (SV), Reissner's membrane (RM), temporal bone (TB).

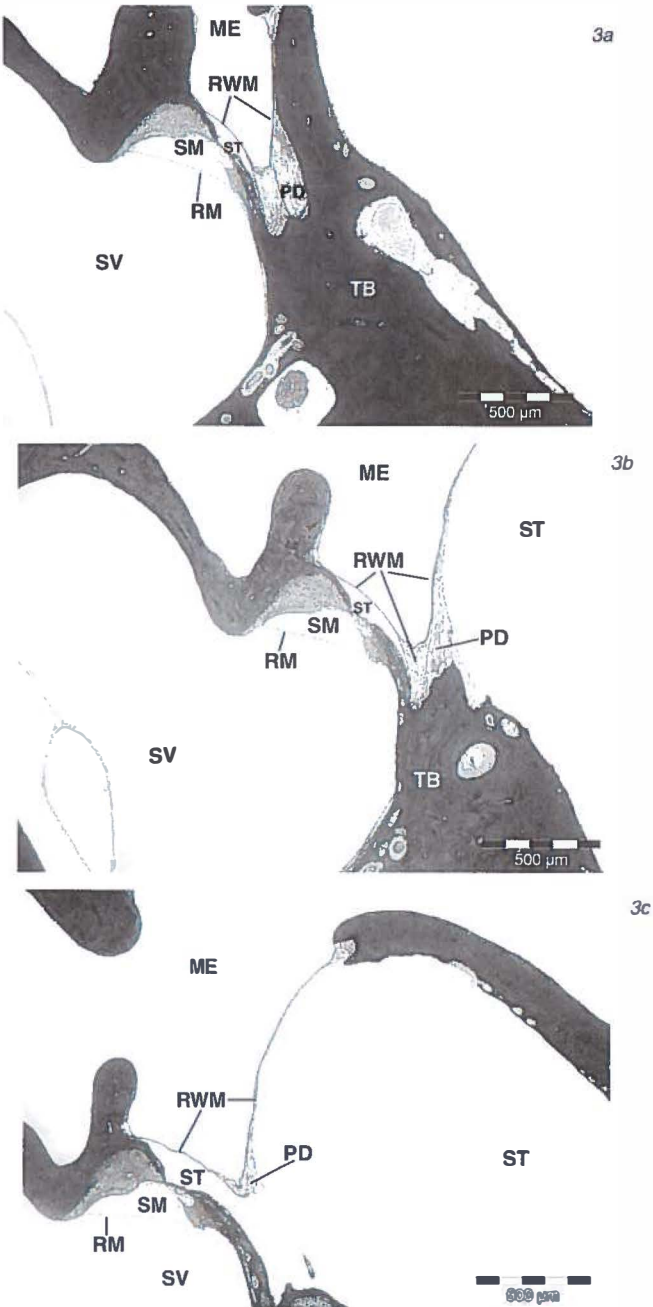


Figure 3a, b, c - Longitudinal cross-sections in lateral direction of the connection of the periotic duct (PD) with the medial part of the round window membrane (RWM). Middle ear (ME), scala vestibuli (SV), scala media (SM), Reissner's membrane (RM), temporal bone (TB).

There is no actual barrier membrane between the lumen of the cochlear aqueduct and the perilymphatic space, the lumen of the cochlear aqueduct is contiguous with the perilymphatic space. The fibroblasts and connective tissue trabeculae inside the cochlear aqueduct are arranged more compactly at the opening to the perilymphatic space than in the duct portion (Figure 2). Within the lumen of the cochlear aqueduct, also at the level where the cell meshwork is more compact, multiple intraluminal spaces can be seen between the fibroblasts. At the opening with the scala tympani, part of the fibroblasts and connective tissue trabeculae end at the bony side of the cochlear aqueduct. The other part of the periotic duct is connected with the medial limit of the round window membrane (Figure 3a-c).

The round window membrane has a pouch-like extension, with the concave side at the middle ear cavity. At the side where the periotic duct is connected with the round window membrane, the cell meshwork follows the round window membrane into the scala tympani, finally ending in a thin one cell-layer attached to the round window membrane (Figure 3c). At the convex side of the pouch-like extension, the round window membrane appears to become a multi cell-layer structure of approximately 100 μm diameter. This part of the round window membrane, which is about 400 μm in length, follows its course in close connection with the periotic duct at its most cell dense part. The extension of the round window membrane does not enter the cochlear aqueduct, but has a location of fixation at the temporal bone which is an excavation adjacent to the cochlear aqueduct entrance (Figure 4a-b).

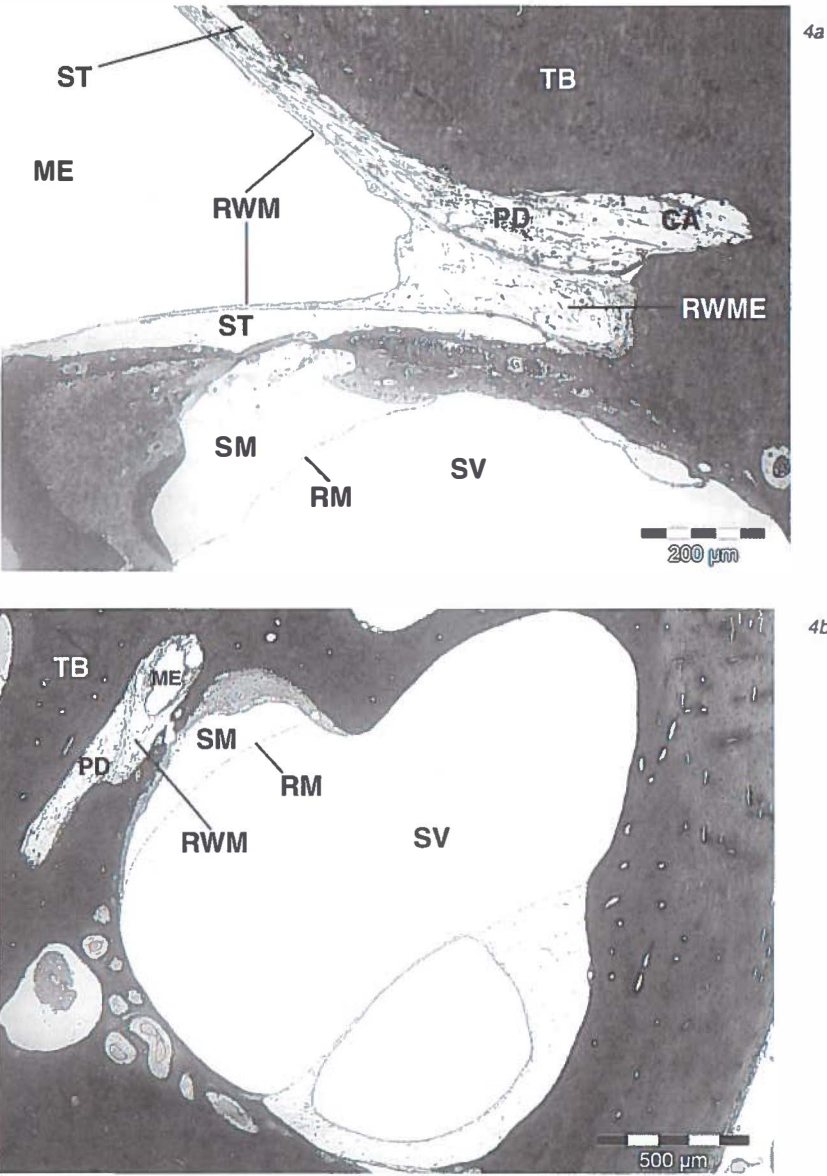


Figure 4a, b - Longitudinal (a) and perpendicular (b) cross section of the multi cellular pouch-like extension (RWME) of the round window membrane (RWM), connected with an excavation of the temporal bone next to the entrance of the cochlear aqueduct (CA), in close relation with the periotic duct (PD). Scala vestibuli (SV), scala media (SM), scala tympani (ST), Reissner's membrane (RM), middle ear (ME), temporal bone (TB).

Discussion

The observation of the flattened funnel shape and varying diameter of the cochlear aqueduct is in accordance with literature (Nishimura et al. 1981; Yoshida and Uemura 1991; Ghiz et al. 2001). In the past some researchers reported the presence of a 'barrier membrane' between the cochlear aqueduct and the perilymphatic space (Waltner 1948; Palva and Dammert 1969; Keleman et al. 1979). Waltner (1948) named this so-called barrier membrane the *Membrana Limitans*. We could not confirm the existence of such a structure the internal orifice of the cochlear aqueduct, neither did Nishimura (1981). It is possible that in thick sections, such as serial sections of celloidin-mounted specimens, a pseudomembranous structure may sometimes be observed at the opening of the duct, due to the density and the arrangement of the fibroblasts at this site (Waltner used 10 μm thick sections).

The periotic duct tissue within the lumen of the cochlear aqueduct, composed of reticular cells and collagen and elastic fibrils, forms an intricate cell-meshwork characterized by dense and sparse portions. At the opening to the scala tympani the arrangement of these cellular components is more compact than in the duct portion. The periotic duct extends into the scala tympani and is connected with the medial part of the round window membrane. This connection is in accordance with the observations of Yoshida (1991) and Nishimura (1981).

The cochlear aqueduct is the main pressure transduction canal connected with the inner ear (Beentjes 1972; Marchbanks and Reid 1990; Carlborg et al.). However, the fluid flow through the cochlear aqueduct is not completely free. The aqueduct has a variable flow resistance (Wit et al. 1999, 2003; Thalen et al. 2001, 2002). In previous studies we calculated the flow resistance of the cochlear aqueduct following inner ear pressure changes caused by manipulation of CSF pressure (Thalen et al. 2001), external ear canal pressure (Thalen et al. 2002) or by injection or withdrawal of fluid directly from the scala tympani (Wit et al. 2000). In these experiments the flow resistance of the cochlear aqueduct depended both on the direction of the fluid flow through the aqueduct and on the pressure gradient across the cochlear aqueduct. Flow resistances for fluid flow out of the inner ear were smaller than the flow resistances for fluid flow in the opposite direction. The

higher the inner ear pressure the smaller the flow resistance. This variable flow resistance cannot be explained by the bony structure of the cochlear aqueduct itself. The observations in this study and in earlier reports indicate a connection of the periotic duct as part of the cochlear aqueduct both with the subarachnoid membrane and the round window membrane (Toriya et al. 1991; Nishimura et al. 1981). Especially its connection with the round window membrane might be relevant for the influence of middle and inner ear pressure on the flow resistance of the cochlear aqueduct. The round window membrane, which is three times as compliant as the oval window membrane (Ivarsson and Pedersen 1977; Ishii et al. 1995), has a position that depends on middle and inner ear pressure. The pouch-like extension of the round window membrane is a multi cell-layer structure with a length of approximately 400 μm and a diameter of approximately 100 μm at its connection with the temporal bone. This part of the round window membrane is adjacent to the most cell dense part of the periotic duct where the fibroblasts are arranged more perpendicular, at the internal orifice of the cochlear aqueduct. An increase of inner ear pressure (or a decrease of middle ear pressure) will cause an outbulging of the round window membrane into the middle ear and stretching of the periotic duct that might increase its permeability. A decrease of inner ear pressure causes the window membrane to move in the opposite direction, followed by a decrease of the permeability of the CA. The cell dense part of the pouch like extension of the round window membrane might be compressing the periotic duct at its most cell dense part.

This variable position of the round window membrane might explain the variable flow resistance of the cochlear aqueduct, and the pressure dependency of its flow resistance. The meshwork of periotic duct tissue in close relation with the position of the round window membrane, functions to regulate sudden fluid pressure changes in the duct which may arise from both the middle ear, the inner ear and the cerebrospinal fluid compartment. This mechanism might protect the inner ear against sudden changes of pressure.

Chapter 8

SUMMARY AND CONCLUSIONS

Summary

Adequate functioning of the inner ear pressure regulation mechanism is a prerequisite for normal functioning of the inner ear. Although inner ear pressure is constantly changing, these pressure fluctuations do not affect the inner ear functions of hearing and maintenance of spatial equilibrium in healthy humans. Disregulation of inner ear pressure can cause clinical symptoms, such as a fluctuating hearing loss, vertigo, tinnitus or aural fullness. The regulation of inner ear pressure is a complicated process which is not fully understood to date. Inner ear pressure changes can be corrected by means of fluid transport through the cochlear aqueduct. Although the cochlear aqueduct has been subject of extensive research, very few studies have been performed concerning its dynamic properties for fluid transport; quantitative details concerning these properties are scarce. By means of fundamental experimental inner ear pressure research, increase of our knowledge concerning the process of inner ear pressure regulation will provide a better insight in the physiology and pathophysiology of the inner ear. Using a servo-controlled micropipette system (WPI 900A micro pressure system), we invasively measured inner ear pressure, both in scala tympani and scala media during various ways of inner ear pressure manipulation, and studied the dynamics of pressure recovery. As this invasive measurement technique is not suitable for human ears, the experiments were performed in guinea pigs. The obtained physiological results were related to the morphology of the cochlear aqueduct in a light microscopic study.

In Chapter 2 the anatomy of the labyrinth is described, and the composition and dynamic behaviour of the inner ear fluids, as well as the hydrostatic pressure of the inner ear, are discussed. Furthermore the perilymphatic communication routes with the cerebrospinal fluid compartment are described, with emphasis on the cochlear aqueduct.

Hydrostatic pressure of the inner ear is determined by multiple variables, of which the most relevant are: the production and absorption of inner ear fluids, cerebrospinal fluid pressure, middle ear pressure and the elastic properties of the membranous labyrinth. The perilymphatic compartment of the inner ear is connected with the cerebrospinal fluid compartment through several routes: the cochlear aqueduct, Hyrtl's fissure, perivascular – and perineural canals.

The cochlear aqueduct is the main pressure transduction canal (by means of fluid flow) connected with the inner ear. Inside its lumen is the periotic duct, a meshwork of fibroblasts and connective tissue.

In Chapter 3 the dynamics of inner ear pressure release are discussed, following inner ear pressure manipulation in the endolymphatic compartment. In 8 guinea pigs inner ear fluid pressure was measured in scala media through one barrel of a double-barrelled micropipette after a sudden volume increase or decrease, caused by injection or withdrawal of (artificial) endolymph through the other barrel. How a volume change affects the inner ear pressure depends on the compliance of the bordering membranes of the inner ear and the flow resistance of the pressure release routes (the inner ear is a leaky system). During injection or withdrawal, the inner ear pressure changed in the order of 1-10 cm water, but it returned to its initial value within a few seconds. The obtained results could be fitted with a physical model. In this model the inner ear was depicted by a rigid cavity, because it is surrounded by bone, except for the window membranes, which were represented by a compliance C . From the inner ear cavity fluid can escape through an escape route (cochlear aqueduct), which was represented by a flow resistance R . A single exponential function, in which volume flow was assumed to be proportional to pressure, a *linear* process, did not fit well to our experimental results. A double exponential function in which it was assumed that inner ear pressure recovery is a *nonlinear* process, governed by a pressure dependent flow resistance and/or membrane compliance, gave perfect fits to most of our experimental results. From these fits a time constant (the time interval in which the pressure returns from its maximum value to e^{-1} times this maximum value) could be derived. Smaller time constants indicate a faster pressure equalisation. Time constants for the pressure recovery depended on the flow direction. It was on average 1.1 s after a short overpressure and 2.8 s after underpressure.

Chapter 4 presents the dynamics of inner ear pressure change following sudden pressure changes in the cerebrospinal fluid (CSF) compartment. In 14 guinea pigs inner ear pressure was measured in scala tympani during two related CSF pressure manipulation profiles: square waves and short rectangular pulses. Square wave manipulation of CSF pressure was performed with low frequency square pressure steps having a standard

frequency and amplitude in 8 guinea pigs. Pulse-wise manipulation was performed with a standard pulse width (1 sec) and repetition rate (0.05 s^{-1}) and varying amplitudes in 9 guinea pigs. The onset of inner ear pressure change following manipulated CSF pressure was immediate, without a measurable time lag. Inner ear pressure equalised within a few seconds. During square wave CSF pressure manipulation, the inner ear pressure curves had the shape of a low-pass filtered square wave. Like in the previous study, fits with a single exponential function (assuming a constant compliance and a constant flow resistance) showed systematic deviations from the pressure curves. With the more complicated nonlinear model, almost perfect fits to the inner ear pressure equalisation curves could again be obtained. This nonlinearity may be the consequence of dependence of the compliance and/or the flow resistance on pressure. Time constants derived from the fits of the pressure curves following square wave- wise CSF pressure manipulation were averaged 2.8 s following pressure increase, and 2.0 s following pressure decrease. So following manipulated CSF pressure inner ear pressure decreases somewhat faster than it increases. Following the pulse-wise CSF pressure manipulation, mean time constant was 1.7 s. By increase of positive inner ear pressure changes, time constants decreased, indicating a faster pressure equalisation.

In Chapter 5 the dynamics of inner ear pressure change are presented, following square wave-wise pressure manipulation of the CSF compartment and subsequently the external ear canal (EEC) in the same guinea pig. During CSF pressure manipulation the inner ear pressure curves had the shape of a low-pass filtered square wave. Mean inner ear pressure change was 1.4 cm water. Mean time constant following pressure increase was 3.2 s (fluid flow into the cochlea), for pressure decrease 2.3 s (fluid flow out of the cochlea). During EEC pressure manipulation the inner ear behaved as a complementary high-pass system: a pressure change in the EEC is immediately transferred to the inner ear through the oval window; inner ear pressure subsequently slowly returns to the equilibrium value because fluid escapes through the cochlear aqueduct. Mean maximum pressure step in the inner ear was 3.6 cm water, with a mean time constant of 1.72 s (fluid flow out of the cochlea); mean maximum negative pressure change of the inner ear was 2.4 cm water, with a mean time constant of 2.85 s (fluid flow into the cochlea). This asymmetric high-pass filtered pressure response was obvious

in all experiments, except for the very small EEC pressure changes. Best fits to the pressure equalisation curves were made with a nonlinear model for pressure equalisation, indicating a variable and probably pressure depended flow-resistance of the cochlear aqueduct and/or compliance of the membranous labyrinth. Time constants for pressure changes during CSF or EEC pressure changes were comparable, but not equal. Time constants were in the order of seconds and were similar in their dependence on the direction of flow through the cochlear aqueduct. Fluid flow out of the cochlea is somewhat faster than flow in the opposite direction. An explanation for this might be found in the special structure of the periotic duct inside the cochlear aqueduct.

In Chapter 6 the results are presented of a series of experiments in 6 guinea pigs in which the flow resistance of the cochlear aqueduct is measured. In our previous experiments we find a variable, pressure dependent time constant derived from the fits made of the nonlinear pressure equalisation curves of inner ear pressure changes following CSF and/or EEC pressure manipulation. This pressure dependency was determined either by the flow resistance of the cochlear aqueduct, or by the compliance of the membranous labyrinth (especially the round window membrane), or by their combination. This study was designed to determine the flow resistance of the cochlear aqueduct separately. We ruled out the possible influence of the compliant nature of the membranous labyrinth by a constant infusion or withdrawal of (artificial) perilymph into or from the scala tympani at various rates until new steady state pressures were achieved. In such a situation the injection or withdrawal rate is equal to the rate of fluid flow through the cochlear aqueduct. For different steady state pressures of the inner ear, the flow resistance of the cochlear aqueduct was calculated. The flow resistance was not constant, but depended on inner ear pressure, the flow direction through the cochlear aqueduct and possibly the flow magnitude. During increased inner ear pressure the round window membrane bulges outwards, and the flow resistance of the cochlear aqueduct decreases. During decreased inner ear pressure the window membrane moves in the opposite direction, in combination with an increase of the flow resistance. The periotic duct inside the cochlear aqueduct is connected with the round window membrane, its structure and permeability might be influenced by the position of the round window membrane. With an estimation of the compliance of the membranous

labyrinth (0.17 nl/Pa), and a given range of time constants (0.9-5.4 s), the flow resistance of the cochlear aqueduct varied between 5 and 32 Pa.s/nl. For very small flow values the averaged flow resistance was 21 Pa.s/nl.

Chapter 7 contains the results of a light microscopic study of the cochlear aqueduct in 5 guinea pigs, with emphasis on its relation with the round window membrane. This detailed morphological information of the cochlear aqueduct can contribute to a further understanding of its dynamic properties. The flow resistance of the cochlear aqueduct depends on the fluid flow direction, inner ear pressure and the pressure gradient across the aqueduct. The cochlear aqueduct itself is a funnel shaped bony canal with an open connection with the scala tympani, which can not explain its dynamic characteristics. Inside the cochlear aqueduct runs the periotic duct, a meshwork of fibroblasts and connective tissue. The fibroblasts and connective tissue trabeculae inside the cochlear aqueduct are arranged more compactly at the opening to the perilymphatic space than in the duct portion. Part of the periotic duct is connected with the medial limit of the round window membrane. The round window membrane has a pouch like extension with the concave side at the middle ear cavity. At the convex side of the extension, the round window membrane becomes a multi cell-layer structure of approximately 100 μm diameter and 400 μm in length. This cell dense window extension runs parallel to the periotic duct at it most cell dense part, and is fixated at an excavation of the temporal bone adjacent to the cochlear aqueduct entrance. The connection of the periotic duct with the round window membrane implicates a role of the pressure dependent position of the round window membrane and the permeability of the cochlear aqueduct. Outbulging of the round window membrane into the middle ear causes stretch on the periotic duct and a decrease of the flow resistance. Movement in the opposite direction causes an increase of the flow resistance, possibly by mechanical compression of the most cell dense part of the periotic duct. This structural feature suggests that the inner ear can be protected against sudden pressure changes by the variable flow resistance of the cochlear aqueduct.

Conclusions

The complex physiology of the labyrinth has not been unravelled yet. Disregulation of inner ear pressure is thought to be related to clinical

symptoms in man, such as tinnitus, vertigo attacks, aural fullness or fluctuating hearing loss. In our study we could quantify the process of inner ear pressure change, by calculating a time constant from fits made to the measured inner ear pressure recovery curves after induced pressure changes. Inner ear pressure recovery following both direct inner ear pressure manipulation by the infusion of artificial endolymph into scala media, and indirect inner ear pressure manipulation by changing pressure in the cerebrospinal fluid compartment or the external ear canal, turned out to be nonlinear.

Our experiments indicate that this nonlinearity is caused by the pressure dependent flow resistance of the cochlear aqueduct. A varying position of the round window membrane might cause the varying permeability of the cochlear aqueduct. With light microscopic images we have shown that the periotic duct (the tissue within the bony cochlear aqueduct) is connected with the round window membrane. An increase of inner ear pressure will cause an outbulging of the round window membrane into the middle ear and stretching of the periotic duct. This might increase the permeability of the cochlear aqueduct, thus facilitating pressure equalization in case of an increased inner ear pressure.

Further research concerning the interesting and relevant mechanism of inner ear pressure regulation is a prerequisite to develop relevant tools for diagnosis and treatment in clinical practice.

Samenvatting

Het binnenoor bestaat uit het gehoororgaan en het evenwichtsorgaan. Het gehoororgaan, de cochlea, wordt ook wel het slakkenhuis genoemd vanwege zijn vorm. De cochlea bestaat uit een aantal windingen. Deze windingen zijn onderverdeeld in drie compartimenten: scala tympani, scala vestibuli en scala media. Deze compartimenten zijn gevuld met twee soorten binnenoorvloeistof. Scala tympani en scala vestibuli zijn gevuld met perilymfe, scala media is gevuld met endolymfe. In scala media bevindt zich het zintuigorgaan van het binnenoor: het orgaan van Corti.

Voor het goed functioneren van het binnenoor is een juiste drukregulatie ervan een vereiste. De binnenoordruk bij mensen verandert voortdurend, bijvoorbeeld bij bukken, hoesten en ademen. De functies van het binnenoor raken niet verstoord, ondanks deze dagelijkse fysiologische drukveranderingen van het binnenoor. Een verstoring van de drukregulatie van het binnenoor kan klinische symptomen veroorzaken, zoals een wisselend perceptief gehoorverlies, draaiduizeligheid, oorsuizen of een drukgevoel in het oor.

Een drukverandering van het binnenoor kan snel worden gecorrigeerd door een in- of uitstroom van vloeistof door de *aqueductus cochlearis*. Deze aqueductus cochlearis is een klein botkanaaltje dat het binnenoor verbindt met de intracraniële ruimte. In het verleden is uitgebreid onderzoek verricht naar de aqueductus cochlearis. Er zijn echter weinig studies waarbij ook werd gekeken naar de dynamische eigenschappen van dit kanaaltje. Door onderzoek te verrichten naar het mechanisme voor drukregulatie van het binnenoor zal een beter inzicht kunnen worden verkregen in de fysiologie, en daardoor ook in de pathofysiologie, van het binnenoor.

Wij hebben bij cavia's (het meest gebruikte proefdiermodel voor het binnenoor van de mens) op invasieve wijze de binnenoordruk gemeten. Door het ronde venster werd een elektrode in scala media of scala tympani geplaatst. Deze elektrode was verbonden met een apparaat dat kleine drukveranderingen in zeer kleine ruimtes kan meten. Vervolgens werd de binnenoordruk direct en indirect gemanipuleerd en werd het proces van drukverandering, met name het proces van drukherstel van het binnenoor, bestudeerd.

In Hoofdstuk 2 wordt de anatomie van het binnenoor beschreven, evenals de samenstelling en fysiologie van de binnenoorvloeistoffen. Het perilymfatische compartiment van het binnenoor en het intracraniale compartiment, dat gevuld is met cerebrospinale vloeistof, zijn onderling verbonden door verschillende botkanaaltjes (het binnenoor is een lek systeem). De aquaductus cochlearis is het belangrijkste verbindingskanaaltje. Door een vloeistofstroom door de aquaductus, het oor in of het oor uit, kan de druk in het binnenoor veranderen. In het lumen van de aquaductus cochlearis bevindt zich een losmazig netwerk van fibroblasten en bindweefsel, de zogenaamde *ductus perioticus*.

In Hoofdstuk 3 wordt de dynamiek van drukveranderingen van het binnenoor bestudeerd, nadat de druk in het endolymfatische compartiment rechtstreeks is gemanipuleerd. Met een dubbele micropipet werd scala media aangeprikt. Door de ene pipet werd de druk gemeten, terwijl door de andere pipet (kunstmatige) endolymfe werd opgezogen of ingespoten. De mate waarin een volumeverandering de binnenoordruk kan beïnvloeden wordt mede bepaald door de compliantie van de membranen die het binnenoor begrenzen en de stromingsweerstand van de kanaaltjes verbonden met het binnenoor, door welke een vloeistofstroom mogelijk is. Na een drukverandering herstelde de binnenoordruk zich binnen enkele seconden.

Van de verkregen resultaten werden fits gemaakt met een functie die drukveranderingen in binnenoor beschrijft. Deze functie is afgeleid van een eenvoudig model van het binnenoor als drukcompartiment, afgesloten door een elastisch membraan. Een functie waarbij er vanuit werd gegaan dat een volumestroom het binnenoor in of uit recht evenredig was met de mate van drukverandering (een lineaire functie) kwam niet goed overeen met onze meetresultaten. Een veel betere weergave werd verkregen met een functie waarbij er van werd uitgegaan dat het proces van drukherstel niet lineair was. Bij deze niet-lineaire functie werd er van uitgegaan dat er sprake was van een drukafhankelijke stromingsweerstand, of een drukafhankelijke compliantie van structuren die het binnenoor begrenzen. Om deze niet-lineaire functie zo passend mogelijk te maken bij de verschillende meetresultaten, werden de afzonderlijke metingen gefit. Van een dergelijk fit kon vervolgens een tijdsconstante worden afgeleid. Hoe kleiner een tijdsconstante, hoe sneller het proces van de drukverandering.

Tijdsconstanten voor drukverhoging (vloeistofstroom het oor uit) en drukverlaging (vloeistofstroom het oor in) waren verschillend. Tijdsconstanten voor drukverhoging waren gemiddeld 1.1 s, terwijl de tijdsconstanten voor drukverlaging gemiddeld 2.8 s waren.

In Hoofdstuk 4 wordt de drukverandering van het binnenoor gemeten, na manipulatie van de druk in de cerebrospinale ruimte (CSF-druk). De CSF-druk werd puls- en blokvormig gemanipuleerd. De blokvormige drukmanipulatie werd uitgevoerd met een standaard frequentie en standaard amplitude. De pulsvormige drukmanipulatie werd uitgevoerd met een standaard duur en frequentie, en een variabele amplitude.

De binnenoordruk volgde zonder een meetbare vertraging de (gemanipuleerde) CSF-druk. Gedurende de blokvormige drukmanipulatie had de binnenoordruk het model van een *low-pass* gefilterd druksignaal. Net als in het vorige hoofdstuk werden fits gemaakt aan de drukmetingen. Ook hier bleek dat een fit met de niet-lineaire functie bijna perfect overeenkwam met de meetresultaten, in tegenstelling tot fits met de lineaire functie. Tijdsconstanten voor de fits gedurende verhoging van de CSF-druk (vloeistofstroom het oor in) waren gemiddeld 2.8 s, terwijl tijdsconstanten voor de fits tijdens verlaging van de CSF-druk (vloeistofstroom het oor uit) gemiddeld 2.0 s waren.

Gedurende de pulsvormige drukmanipulatie was de gemiddelde tijdsconstante voor het proces van drukherstel (vloeistofstroom het oor uit) 1.7 s. Bij toename van de amplitude van de drukverandering werden de tijdsconstanten kleiner. Dit betekent dat de binnenoordruk zich sneller herstelt na een grotere drukverhoging.

In Hoofdstuk 5 wordt de dynamiek van drukveranderingen van het binnenoor bestudeerd na blokvormige manipulatie van in eerste instantie de CSF-druk en vervolgens de druk in de uitwendige gehoorgang, bij dezelfde cavia. Gedurende manipulatie van de CSF-druk had het gemeten druksignaal van het binnenoor de vorm van een *low-pass* gefilterde drukgolf. De gemiddelde tijdsconstante na een drukverhoging (vloeistofstroom het oor in) was 3.2 s, de gemiddelde tijdsconstante na een drukverlaging (vloeistofstroom het oor uit) was 2.3 s.

Gedurende drukmanipulatie in de uitwendige gehoorgang had de gemeten binnenoordruk het model van een *high-pass* gefilterde drukgolf: een drukverandering in de uitwendige gehoorgang wordt direct doorgevoerd naar het binnenoor via de vensters; de binnenoordruk herstelt vervolgens door een vloeistofstroom het oor in of uit door de aquaductus cochlearis. De maximale positieve druksprong van het binnenoor was gemiddeld 3.6 cm water, met een tijdsconstante van 1.72 s (vloeistofstroom het binnenoor uit). De maximale negatieve druksprong was gemiddeld 2.4 cm water, met een tijdsconstante van 2.85 s (vloeistofstroom het binnenoor in). Dit asymmetrische druksignaal zagen wij in alle experimenten, behalve bij heel kleine drukveranderingen. Wederom werden de meest nauwkeurige fits verkregen met de niet-lineaire functie. De tijdsconstanten voor de drukveranderingen gedurende manipulatie van de CSF-druk of drukmanipulatie in de uitwendige gehoorgang waren vergelijkbaar, maar niet identiek. Overeenkomst tussen de tijdsconstanten was de orde van grootte (enkele seconden), en hun relatie met de richting van de vloeistofstroom door de aquaductus cochlearis. Een vloeistofstroom het binnenoor uit ging iets sneller dan een vloeistofstroom in tegengestelde richting. Een verklaring hiervoor zou de speciale structuur van de ductus perioticus kunnen zijn.

In Hoofdstuk 6 worden de resultaten gepresenteerd van een serie experimenten waarbij de stromingsweerstand van de aquaductus cochlearis wordt gemeten.

In onze voorgaande experimenten vonden we een drukafhankelijke tijdsconstante voor het proces van drukherstel van het binnenoor. Deze drukafhankelijkheid van de tijdsconstante zou bepaald kunnen worden door óf de stromingsweerstand van de aquaductus cochlearis, óf de compliantie van het membraaneuze labrynt (met name het ronde venster), óf door een combinatie van beiden.

Bij deze serie experimenten schakelden we de mogelijke invloed van een variabele compliantie van het membraaneuze labrynt uit. Met verschillende snelheden werd gedurende constante infusie (of opzuiging) van kunstmatige perilymphe in (of uit) scala tympani een veranderde stabiele binnenoordruk bereikt. Als tijdens constante infusie (of opzuiging) van vloeistof het binnenoor in (of uit) de druk van het binnenoor stabiel blijft is er sprake van een

evenwicht waarbij de vloeistofstroom het oor uit (of in) door de aquaductus cochlearis gelijk is aan de stromingssnelheid van de infusie (of opzuiging). Bij verschillende binnenoordrukken werd zo de stromingsweerstand van de aquaductus cochlearis gemeten. De stromingsweerstand was niet constant, maar was afhankelijk van de druk in het binnenoor, de stromingsrichting door de aquaductus cochlearis en de mate van drukverandering. Bij een geschatte compliantie van het membraneuze labyrint (0.17 nl/Pa) en een bekende spreiding van tijdsconstanten ($0.9\text{-}5.4 \text{ s}$), varieerde de stromingsweerstand tussen 5 en 32 Pa.s/nl . Gedurende zeer kleine stromingssnelheden was de gemiddelde stromingsweerstand van de aquaductus cochlearis 21 Pa.s/nl .

Gedurende een toename van de binnenoordruk zal het ronde venster uitbollen in de richting van het middenoor. In deze situatie is er sprake van een kleinere stromingsweerstand van de aquaductus cochlearis. Gedurende een afname van de binnenoordruk zal het ronde venster zich verplaatsen in tegengestelde richting. In deze situatie is er een toename van de stromingsweerstand van de aquaductus cochlearis. De ductus perioticus, welke zich in het lumen van de aquaductus cochlearis bevindt, staat in verbinding met het ronde venster. Het is mogelijk dat de structuur en de permeabiliteit van de ductus perioticus worden beïnvloed door de stand van het ronde venster. Dit zou de drukafhankelijkheid van de stromingsweerstand van de aquaductus cochlearis kunnen verklaren.

In Hoofdstuk 7 worden de resultaten gepresenteerd van een studie d.m.v. lichtmicroscopie van de aquaductus cochlearis. Hierbij is vooral gekeken naar de verbinding van de aquaductus met het binnenoor en naar de relatie van de aquaductus en de ductus perioticus met het ronde venster. De aquaductus cochlearis is een klein benig trechtervormig kanaaltje. De aquaductus mondt uit in scala tympani. De structuur van dit benige kanaal kan niet de drukafhankelijke stromingsweerstand verklaren. De ductus perioticus in het lumen van de aquaductus cochlearis bestaat uit een netwerk van fibroblasten en bindweefselcellen. Deze cellen zijn op de overgang van de aquaductus naar het binnenoor compacter gerangschikt, in verhouding tot de cellen dieper in de aquaductus gelegen. Een deel van de ductus perioticus is verbonden met het mediale deel van het ronde venster. Het ronde venster heeft de vorm van een trechter, waarbij de concave zijde het middenoor begrenst. Aan de convexe zijde van het venster (de punt van de trechter) eindigt het ronde

venster in een compacte structuur van meerdere cellagen met een diameter van ongeveer 100 μm en een lengte van ongeveer 400 μm . Deze extensie van het ronde venster loopt parallel aan het meest celrijke deel van de ductus perioticus, en zit gefixeerd aan de os temporale, vlak naast de uitmonding van de aquaductus in scala tympani. De verbinding van de ductus perioticus met het ronde venster impliceert een relatie tussen de drukafhankelijke stand van het ronde venster en de stromingsweerstand van de aquaductus cochlearis. Immers, een uitbolling van het ronde venster, richting het middenoor, veroorzaakt tractie aan de ductus perioticus en daarmee een afname van de stromingsweerstand van de aquaductus. Een verplaatsing van het venster in tegengestelde richting veroorzaakt een toename van de stromingsweerstand, mogelijk door mechanische compressie van het meest celrijke deel van de ductus perioticus. Dit mechanisme verklaart een variabele stromingsweerstand van de aquaductus cochlearis, die het binnenoor zou kunnen beschermen tegen plotselinge drukveranderingen.

Conclusie

De fysiologie van het binnenoor is tot op heden nog niet volledig doorgrond. Bij een verstoring van de drukregulatie van het binnenoor kunnen klinische symptomen optreden, zoals een fluctuerend perceptief gehoorverlies, draaiduizeligheid, oorsuizen of een drukgevoel in het oor. In dit onderzoek hebben we het proces van drukverandering van het binnenoor gekwantificeerd, door hiervoor een tijdsconstante af te leiden. Het proces van drukherstel van het binnenoor bleek niet lineair te zijn, zowel na directe manipulatie middels infusie van kunstmatige endolympe in scala media, als na indirecte manipulatie middels drukverandering in de CSF-ruimte of de uitwendige gehoorgang. Onze experimenten tonen aan dat deze niet-lineariteit wordt veroorzaakt door een drukafhankelijke stromingsweerstand van de aquaductus cochlearis. Met lichtmicroscopische beelden hebben we getoond dat de ductus perioticus vast zit aan het ronde venster. Bij een toename van de binnenoordruk zal het ronde venster uitbollen richting middenoor, waardoor middels tractie aan de ductus perioticus de stromingsweerstand van de aquaductus cochlearis zal afnemen. Op deze wijze zal de binnenoordruk zich sneller kunnen herstellen.

Deze conclusie is gebaseerd op resultaten verkregen bij proefdieronderzoek.

Verder onderzoek is nodig om het interessante en relevante mechanisme van de binnenoordrukregulatie te kunnen doorgronden, opdat in de toekomst patiënten, die lijden aan klinische symptomen veroorzaakt door een disregulatie van de binnenoordruk, beter geholpen kunnen worden.

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Abbreviations

CA	cochlear aqueduct
CSF	cerebrospinal fluid
EEC	external ear canal
EP	endolymphatic potential
Kopje 1-4	koffie compleet
ME	middle ear
PD	periotic duct
RM	Reissner's membrane
RWM	round window membrane
RWME	round window membrane extension
SM	scala media
ST	scala tympani
SV	scala vestibuli
TB	temporal bone

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Curriculum Vitae

The author of this thesis, Elisabeth O. Laurens-Thalen, was born on June 5th of 1970 in Groningen. After graduation from high school (Gymnasium-β at the Praedinius Gymnasium in Groningen) in 1988, she studied medicine at the University of Leiden, the Netherlands, until 1996. From July 1996 she started research in preparation of this thesis as well as clinical work at the Department of Otorhinolaryngology / Head and Neck surgery at the University Hospital Groningen. In October 1998 she started her residency in the same department (Prof. Dr. F.W.J. Albers), in part at the Isala Klinieken Zwolle (Dr. H.J. Rosingh). Her residency was finished in December 2003. Currently, she works as an ENT-surgeon at the Department of Otorhinolaryngology / Head and Neck surgery of the University Hospital Groningen.

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